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EFFECTS OF RADIOACTIVE PHOSPHORUS (P^{32}) ON NORMAL TISSUES

A Histologic Study of the Changes Induced in the Organs of Patients with Malignant Lymphomas

WILLIAM R. PLATT, M.D.
ST. LOUIS

RADIOPHOSPHORUS has been used for the last seven years in the experimental and clinical study of various blood dyscrasias, especially primary polycythemia and the leukemias. Although much has been written concerning the distribution and deposition of this radioactive element in the peripheral blood and body tissues of certain laboratory animals and individual patients, few reports [1] have been made on the histologic changes induced by this type of beta particle radiation. Warren and Cowing [1a] have studied the effects of the intraperitoneal inoculation of radioactive phosphorus on the liver, voluntary muscle, bone, spleen and kidneys in rats. This scarcity of articles on the histologic effects of radiophosphorus is probably related to the fact that the biologic effects of radiophosphorus are qualitatively similar to those of roentgen radiation—the effects of roentgen rays on normal tissues having been fully described by Warren and his co-workers.[2] Although most of the pathologic changes produced by this temporarily radioactive isotope (P^{32}) are identical with those seen after the application of roentgen radiation, there are certain differences in the mode of administration, the duration of activity and the selective concentration which may serve to explain certain tissue alterations produced by it.

PREPARATION AND USAGE OF RADIOACTIVE PHOSPHORUS

The radiophosphorus used in the patients studied in this series [3] was prepared in the cyclotron of the Mallinckrodt Institute of Radiology by the bombardment of red phosphorus with 12,000,000 volt deuterons (nuclei of the heavy isotope of hydrogen, called deuterium). In this procedure, ordinary stable red

From the Department of Pathology, Washington University School of Medicine.

1. (*a*) Warren, S., and Cowing, R. F., abstracted, Arch. Path. **29**:730, 1940. (*b*) Warren, S., and Gates, O.: ibid. **30**:440, 1940.

2. (*a*) Warren, S.: Arch. Path. **34**:443, 1942. (*b*) Dunlap, C. E., and Warren, S.: ibid. **34**:562, 1942.

3. Reinhard, E. H.; Moore, C. V.; Bierbaum, O. S.; Moore, S., and Kamen, M. D.: J. Lab. & Clin. Med. **31**:107, 1946.

phosphorus, previously smeared on a copper target, is placed directly in the path of a beam of high speed deuterons emitted by a cyclotron. The nuclei of a small fraction of the phosphorus atoms then receive a neutron each as a result of this bombardment. Ordinary stable phosphorus has 15 protons and 16 neutrons, each having a mass of 1—making an atomic mass of 31 for this element. Ordinarily the mass of any atom is written to the right and slightly above the chemical symbol of the atom. Therefore, stable phosphorus is referred to as P^{31}. The atomic mass is increased by 1 when a neutron enters the phosphorus nucleus, and the resultant nucleus has a mass of 32. Radioactive phosphorus is, therefore, referred to as P^{32}; its nucleus is unstable and the atom radioactive. The effect of radioactive phosphorus on tissues is exerted when an electron or beta particle is emitted from the substance. When this occurs, radiophosphorus is transformed into ordinary stable sulfur; i. e., when one of the neutrons in the nucleus changes into a proton, the positive charge on the nucleus is increased by 1 and a new nucleus is formed (sulfur).

The phosphorus, prepared as described, is then synthesized into its dibasic sodium salt,[4] and sufficient distilled water is added to make an isotonic solution (15 mg. Na_2HPO_4 per cubic centimeter). A Lauritsen electroscope (calibrated with a uranium standard) is used to determine the radioactivity of the phosphate solution. The freshly prepared solutions usually showed activity varying between 0.20 and 0.40 millicurie per cubic centimeter. (A millicurie is that amount of any radioactive substance in which 37,000,000 atoms disintegrate per second.[5] Therefore, a microcurie is the amount of any radioactive substance which disintegrates at the rate of 37,000 atoms per second. If 1.1 microcuries of radiophosphorus completely disintegrates inside of a kilogram of tissue, it will deliver to that tissue 1 roentgen equivalent of radiation.) Each dose of radioactive phosphorus, which was given parenterally, varied between 0.1 and 2.5 millicuries (100 and 2,500 microcuries). At first, patients were usually treated two or three times a week. The changes observed in the peripheral blood served to control the therapy. In cases of leukemia the cellular components of the blood were restored to as nearly a normal picture as was possible and an attempt was made to maintain this effect. In the other cases (lymphosarcoma, multiple myeloma, Hodgkin's disease and other diseases), in which there was no elevation of the white blood cell count, radioactive phosphorus was given until peripheral blood changes denoted depression of activity of the bone marrow.

HISTOLOGIC OBSERVATIONS

In this series of 43 cases in which tissue changes produced by radiophosphorus were studied microscopically, the diagnoses were as follows: acute leukemia, 4; chronic leukemia, 21; leukosarcoma, 9; aleukemic leukemia, 1; Hodgkin's disease (lymphogranulomatosis), 2; multiple myeloma, 3; lymphosarcoma, 1; melanoma, 1; and Ewing's sarcoma (angioendothelioma of bone), 1. The ages of the patients with leukemia, who constituted the largest group, varied between 9 and 65 years. Patients with malignant lymphomas who had not been treated with any type of radiation were studied as controls. The gross changes attributable to radiophosphorus observed in various organs were minimal when compared with the alterations demonstrated microscopically throughout most of the tissues examined. The accompanying table shows the radioactivity of the tissues studied in 32

4. Hempelmann, C. H., Jr.; Reinhard, E. H.; Moore, C. V.; Bierbaum, O. S., and Moore, S.: J. Lab. & Clin. Med. **29**:1020, 1944.

5. Low-Beer, B. V. A.; Lawrence, J. H., and Stone, R. S.: Radiology **39**:573, 1942.

Radioactivity of Tissues Obtained at Autopsy *

Patient	Total Dose in Millicuries	Total Duration of Treatment in Weeks	Time in Days from Last Injection of P^{32}	Bone Morrow †	Bone	Liver	Spleen	Kidney	Muscle	Lymph Node	Brain	Lung	Other Organs
Myelogenous Leukemia													
A10915	19.703	74	6	0.064 (1)	.....	0.043 (4)	0.032 (5)	0.048 (3)	0.023 (6)	0.049 (2)			
A11275	69.863	26	60	0.0232 (2)	.....	0.0218 (3)	0.0148 (4)	0.0113 (5)	0.0079 (6)	0.0236 (1)			
A4424J	44.967	52	1	0.294 (1)	0.161	0.218 (3)	0.232 (2)	0.152 (4)	0.098 (5)	.....	.....	.....	0.040 (blood)
A11217	44.236	58	7	0.144 (1)	0.067	0.132 (2)	0.128 (4)	0.080 (3)	0.032 (5)	.....	0.021		
A11195	18.317	22	12	0.0473 (1)	0.00825	0.0470 (2)	0.0279 (4)	0.0305 (3)	0.0216 (6)	0.0278 (5)	.....	.....	0.0069 (blood)
A10959	4.532	1	3	0.263 (1)	.....	0.088 (2)	0.081 (3)	0.061 (5)	0.020 (6)	0.063 (4)			
A11179	13.956	11	26	0.0295 (1)	0.0067	0.0274 (2)	0.0170 (5)	0.0139 (6)	0.0178 (4)	0.0243 (3)			
A11622	21.558	50	10	0.0095 (1)	.....	0.0267 (2)	0.0236 (3)	0.0145 (4)	0.00995 (5)	.....	0.0025		
A11506	19.518	31	33	0.00527 (1)	0.00396	0.00463 (2)	0.00355 (3)	0.00275 (4)	0.00237 (5)	.....	0.0021	0.0065	
A11715	3.470	½	1	0.1180 (1)	0.0523	0.0857 (2)	0.0650 (3)	0.560 (4)	0.0308 (5)				
Lymphatic Leukemia													
A11658	31.440	147	14	0.00905 (2)	.....	0.0108 (1)	0.00624 (5)	0.00681 (4)	0.00482 (6)	0.00886 (3)	0.00362		
A4409J	8.446	5	5	0.084 (5)	0.049	0.101 (1)	0.071 (2)	0.059 (3)	0.043 (4)	.....	.....	0.055	
A11495	23.649	31	21	0.437 (3)	.....	0.0535 (2)	Lost	0.0342 (4)	0.0270 (4)	0.0547 (1)			
A11476	9.364	7	1	0.0064 (4)	0.0032	0.173 (1)	0.152 (2)	0.0886 (5)	0.0447 (6)	0.135 (3)	0.0184		
Leukosarcoma													
A10808	25.800	26	8	0.0297 (4)	.....	0.0486 (2)	0.0504 (1)	0.0408 (3)	0.0249 (5)	.....	0.0120	.....	0.0207 (intestine)
A11788	35.619	103	5	0.0678 (1)	0.0654	0.0515 (3)	0.0525 (2)	0.0371 (4)	0.0141 (6)	0.0338 (5)			
A10754	4.530	3	4	0.0366 (3)	.....	0.0982 (1)	0.0534 (2)	.....	0.0237 (4)	0.0193 (5)	0.0120		
A11063	13.205	70	1	0.145 (1)	0.0025	0.1007 (2)	0.0664 (3)	0.0498 (4)	0.039 (5)				
A11297	17.256	29	8	0.103 (4)	0.165	0.208 (1)	0.180 (2)	0.136 (3)	0.091 (5)	.....	0.050		
A11089	10.088	4	2	0.310 (3)	0.374	0.375 (1)	0.350 (2)	0.280 (4)	0.0856 (6)	0.180 (5)	.....	.....	0.0664 (blood)
A11124	6.858	4	3	0.0504 (4)	0.0126	0.0827 (1)	0.0636 (3)	0.0654 (2)	0.0400 (6)	0.0420 (5)	.....	.....	0.01625 (blood)
A11494	9.560	6	7	0.0520 (3)	.....	0.0670 (1)	0.0443 (4)	0.0434 (5)	0.0190 (6)	0.0559 (2)	.....	0.01875	
Monocytic Leukemia													
A11045	5.210	2	2	0.120 (2)	0.010	0.124 (1)	0.102 (4)	0.090 (5)	0.043 (6)	0.116 (3)	.....	.....	0.103 (blood)
A11408	10.136	8	10	0.0915 (1)	.....	0.083 (3)	0.086 (2)	0.0615 (4)	0.0392 (6)	0.0556 (5)			
A11730	4.964	1	4	0.0620 (2)	.....	0.0535 (3)	0.0340 (1)	0.0332 (5)	0.0263 (6)	0.0474 (4)			
A11579	4.504	½	1	0.000 (2)	.....	0.104 (1)	0.0727 (4)	0.0617 (3)	0.0426 (5)				
Reticulum Cell Sarcoma													
A10797	15.081	18	10	0.0190 (4)	.....	0.0501 (1)	0.0422 (2)	0.0357 (3)	0.0077 (5)	.....	.....	0.0211	0.0003 (tumor mass)
Hodgkin's Disease (Lymphogranulomatosis)													
A10558	11.993	31	24	.....	.....	0.0444	0.008	.....	.....	0.000006			
Multiple Myeloma													
A10698	8.660	7	1	0.1688 (4)	.....	0.3442 (1)	0.1949 (2)	0.1846 (3)	0.0656 (5)	.....	0.0087	.....	0.1087 (tumor), 0.0810 (intestine)
A10645	5.002	2	4	.....	.....	0.1020 (1)	0.0660 (2)	0.0590 (3)					
A11326	10.908	16	61	0.00268 (1)	.....	0.00109 (3)	0.00119 (2)	0.000754 (4)	0.000745 (5)				
Malignant Melanoma													
A11580	7.748	4	5	0.138 (1)	.....	0.0840 (3)	0.0794 (4)	0.0517 (5)	0.0194 (6)	0.0966 (2)	.....	0.0615	0.114 (tumor)

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† The radioactivity is expressed in microcuries per gram of wet tissue, with the relative activity of the various tissues represented by numbers in parentheses. The rating 1 indicates the least activity and the rating 6 the greatest activity.

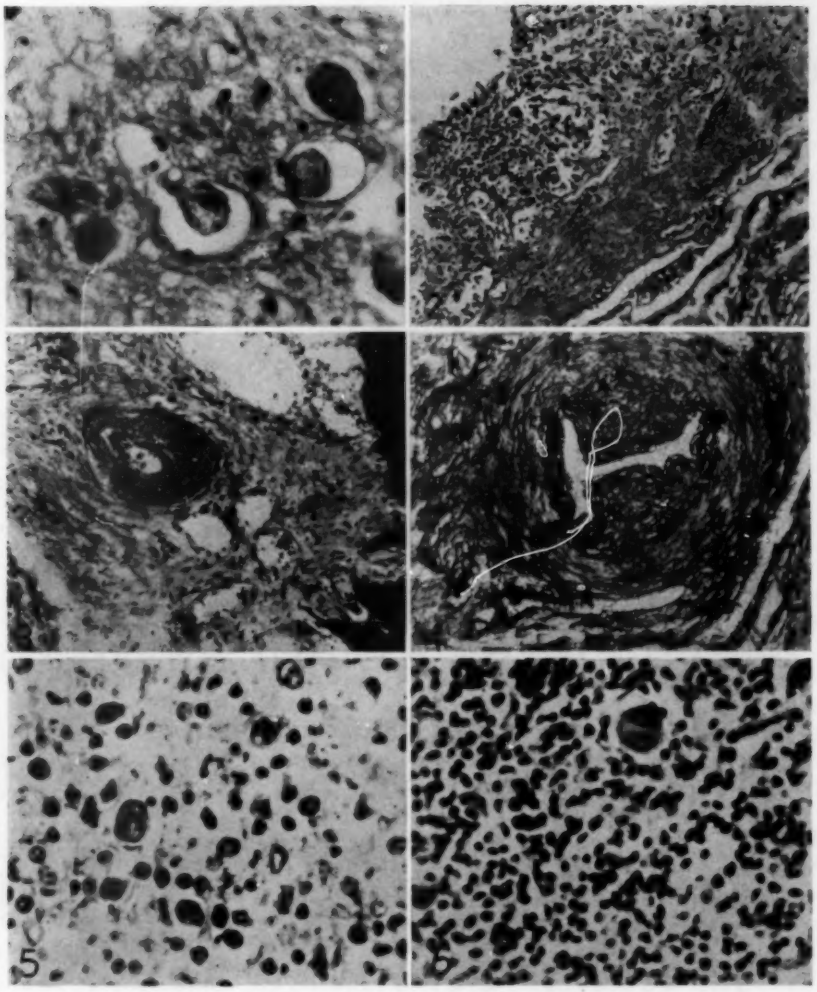

Fig. 1.—Medulla oblongata. Note the eosinophilic outline of neurons showing disappearance of Nissl granules and eccentric location of the pyknotic nuclear remnant. Hematoxylin and eosin stain; × 340.

Fig. 2.—Colon showing atrophy of acini and desquamation of the mucosal epithelium. There is marked hyalinized fibrosis of the mucosa and submucosa, with telangiectasis of vessels in the submucosal layer. Hematoxylin and eosin stain; × 79.

Fig. 3.—Vertebral marrow showing replacement of normal cellular elements by a loose edematous connective tissue containing dilated capillaries. There is marked hyalinization of the medial coat of the artery, with narrowing of its lumen. Hematoxylin and eosin stain; × 79.

Fig. 4.—Uterine artery of 28 year old white woman (no previous pregnancies). The marked proliferation and swelling of the endothelial cells produce thickening of the intima and narrowing of the lumen. Elastic and muscle fibers in the media show fraying and separation with increase in the number of collagen fibers. Hematoxylin and eosin stain; × 79.

Fig. 5.—Lymph node showing large multinucleated atypical giant cells with fused pyknotic nuclei and a rim of basophilic cytoplasm. Hematoxylin and eosin stain; × 340.

Fig. 6.—Lymph node in Hodgkin's disease. Compare the Reed-Sternberg cell with the cells in figure 5 showing multiple, fused, pyknotic nuclei and dark eosinophilic cytoplasm. Hematoxylin and eosin stain; × 340.

of the 43 cases, with the relative activity of the various tissues represented by numbers in parentheses placed to the right of the activity in microcuries. Bone marrow, liver, spleen, kidney, muscle and lymph node were the only six tissues considered in the determination of these relative activities. The rating 1 indicates the greatest activity, whereas the rating 6 indicates the least activity. This distribution of radioactivity was determined by accurately weighing 1 Gm. samples of the fresh tissues removed at autopsy. Digestion with concentrated sulfuric acid and 30 per cent hydrogen dioxide was then individually performed in a Kjeldahl flask. After the clear digest had been diluted with distilled water to a volume of 50 cc., various aliquots of this solution were measured into the glass well of a plunger or a dipping type Geiger counter (Bale tube) until a dilution was found which gave approximately the same number of counts per minute as did a standard radiophosphorus solution of known activity. The radioactivity in microcuries of wet tissue was then calculated from this determination.[3]

Brain.—Permission for an examination of the cerebral tissues was granted in 11 cases, in 8 of which there was a diagnosis of leukemia. In the remaining 3 no metastatic or infiltrative lesions were shown. Hemorrhage and leukemic cell infiltration of the brain, occurring separately or together, were the only significant pathologic changes that could be directly related to the primary disease. However, the nerve cells in the cortex and the white matter situated at a distance from the leukemic alterations showed definite retrogressive changes in a few cases. These consisted of complete disappearance of Nissl granules, loss of nuclei, pyknosis of chromatin matter, swelling and chromatolysis. In some instances mere eosinophilic outlines of former neurons remained (fig. 1). The cerebellum revealed only mild degenerative changes, such as breaking up of cell membranes, loss of Nissl bodies and sometimes disappearance of nuclei. Occasionally foci of neuroglial cell increase were seen, especially in the regions of the cerebrum where the nerve cells were undergoing degeneration. Other changes infrequently noted were perivascular lymphocytic infiltration and meningeal arteriolar hypertrophy with hyalinization of the media and swelling of the intimal coat. In some cases the choroid plexus showed thickening of the villi by an increase of connective tissue in their central cores. The small blood vessels and capillaries in these regions appeared to be somewhat dilated; others were completely obliterated by the sclerotic process. All the preceding changes of glia and stroma were only occasionally observed in the central nervous system; hence it may be concluded that radioactive phosphorus has little effect on nerve tissue. No clinical neurologic observations could be directly or indirectly attributed to these minimal microscopic changes. More than likely, the vascular damage with the associated focal ischemia, rather than direct injury of the nerve elements, is the mechanism of injury in the brain when radiation is applied by isotopic therapy.

Skin.—The epidermis and dermis exhibited pathologic changes which were quite similar to those seen following direct irradiation.[6] Most of the sections examined showed changes ranging from extreme atrophy, disarrangement and disappearance of the basement membrane of epidermal cells to marked hyperkeratosis. There was leveling of the rete pegs, with the cells of the stratum germinativum resting on a hyalinized corium, which contained dense collagen with few elastic fibers. No ulceration was seen. The accessory appendages were atrophic, degenerative and even missing in most of the sections of skin. Only occasional areas showed dilatation of capillaries, arterioles, venules and lymphatics. None of the extreme telangiectatic changes as observed in cases in which erythema and dermatitis were associated with local application of roentgen rays could be demonstrated in these cases of radiophosphorus treatment.

6. Warren, S.: Physiol. Rev. **24**:225, 1944.

Esophagus.—The stratified squamous epithelium covering the mucous membrane of the esophagus reveals the same type of response that was seen in other epithelium-lined internal structures; that is, vacuolation and desquamation of the epithelial cells with edema of the submucosa and infiltrating abnormal blood cells. Fibrosis and thickening of the esophageal musculature were also seen in some instances. In the distal part of the esophagus there were moderate vacuolation of the cells lining the cardiac glands and hypersecretion of mucus. In the evaluation of the aforementioned observations it is necessary to bear in mind that foci of necrosis and subsequent ulceration with its associated chronic inflammatory reaction are frequently found in leukemia, especially in those patients with a low granulocyte count and infection. However, since patients given radiophosphorus treatment present depression of the leukopoietic function of the marrow (see description of bone marrow), with subsequent agranulocytosis and terminal oropharyngeal-esophageal ulceration observed more frequently than in the series of control untreated patients, these conditions may perhaps be caused by the radioactive phosphorus.

Gastrointestinal Tract.—Examination of the gastrointestinal tissues in the 43 cases in this series revealed in 25 the following distribution of lesions which could be attributed to beta ray effect: colon, 9; appendix, 6; small intestines, 6; stomach, 4. Grossly, the intestinal wall was only occasionally thickened and indurated; the serosa was rarely opaque, with moderate telangiectasis. The mucosal epithelial lining showed occasional foci of superficial ulceration (not associated with neoplastic cell infiltration). Regenerating flat to cuboidal epithelium seemed to grow in from the edge of the ulcer, while remnants of the glands within the eroded area sometimes became hyperplastic, with extremely prominent mucous type cells. Those areas of the mucosa which were not ulcerated showed diffuse atrophic changes (fig. 2). Overproduction of mucus by goblet cells, which were enlarged and increased in number, was occasionally seen in distorted glands. The latter were frequently narrowed and elongated or dilated and cystic and were often lined by flattened epithelium. Swollen nuclei with prominent nucleoli were also observed. Occasionally, multinucleated, bizarre, atypical giant cells were seen between the acini and in the submucosal coat proper. Usually accompanying these alterations there was moderate dilatation of lymphatics, capillaries and veins in the mucosa and submucosa. The muscle fibers were the sites of hyaline degeneration, interstitial fibrosis, edema, vacuolation and atrophy.

Liver.—The changes in this organ which could be directly attributed to radiophosphorus were minimal when compared with the more frequently observed alterations in hepatic cells resulting from the anoxia of the severe associated anemia and with the degenerative changes secondary to the leukemic cellular infiltration. Perhaps the most outstanding observation was the variation in size and in chromatism of the hepatic nuclei. There was also fusion of adjacent nuclear structures. The Kupffer cells seemed to enlarge and assumed irregular shapes. There were occasional foci of necrosis, in various stages of degeneration, in the pericentral, midzonal and periportal areas of the hepatic lobule. Some involved the greater part of the lobule and were severe. It is difficult to state specifically whether these changes were secondary to the anoxemia which so frequently accompanies these diseases or whether they were due to the effects of radiophosphorus. There also seemed to be an increased amount of fibrous tissue in the capsule and in the portal regions, with dilatation and slight endophlebitis of some of the radicles of the portal vein. Sclerotic changes

involving the hepatic arteries and proliferative hypertrophy and hyperplasia of the biliary epithelium were minimal.

Bone Marrow.—Sections of vertebral, sternal, rib and femoral marrow were examined. Myeloid hyperplasia was shown in 20 cases; lymphoid hyperplasia and infiltration, with hypoplasia of the other formed elements, in 2; hypoplasia of all the blood cells in 2, and diffuse necrosis and fibrosis of the medullary cavities of both flat and long bones in 18. Macroscopically, the marrow was of a gray to grayish red color and was somewhat less porous than the untreated leukemic or the normal marrow. The histologic changes in the marrow were not completely reflected in the circulating blood. Distributed throughout the hyperplastic marrow there were occasional deposits of hemosiderin, foci of hemorrhage, foci of cell-poor gelatinous edema and regions of fibrosis of varying density, size and maturity. The hypoplastic to aplastic marrows revealed replacement of the normal cellular elements by a loose edematous connective tissue or by a hyalinized collagenous material in which scattered islands of hemopoiesis and rare megakaryocytes survived (fig. 3). In 11 cases large atypical giant cells were observed, many multinuclear and an occasional uninuclear cell.

Lymph Nodes.—In considering the histologic changes observed in lymphoid tissue after the administration of radiophosphorus the cases of Hodgkin's disease, lymphosarcoma, melanoma and Ewing's sarcoma were excluded from consideration, especially with reference to the frequency of occurrence of necrotic and fibrotic alterations. There was complete destruction of pattern in practically all of the leukemic nodes examined. This was usually associated with infiltration of immature myeloid, monocytic and lymphoid cells. The latter type of cells especially showed degeneration of the nuclei (chromatolysis, karyorrhexis and pyknosis). Grossly, most of the nodes were enlarged, moderately firm, grayish white on cut section and nonadherent to the surrounding structures. Moderate to severe collagenous thickening with occasional hyalinization was observed in the capsule in 20 cases. There were telangiectasia of capillaries, lymphatics and venules in 6 cases and associated focal fibrillary eosinophilic necrosis in 4. Atypical giant nuclei (fig. 5) were demonstrated in 20 cases, in 11 of which the nodes contained no areas of necrosis or fibrosis. The nuclear chromatin in these bizarre cells was usually increased in density, and prominent nucleoli with abnormal mitotic figures were also seen. The cytoplasm varied from light basophilic to dark eosinophilic and was nongranular; frequently the nucleus occupied the entire cellular structure. These cells resembled the atypical megakaryocytes produced as a result of irradiation. However, their increased diameter, the absence of light eosinophilic cytoplasm and their presence in other tissues of the body not associated with extramedullary hemopoiesis make this presumption somewhat doubtful. It seems more likely that they represent variants of reticulum cells, fibroblasts or immature myeloid elements produced as a result of multipolar mitoses. However, the possibility that they are produced by fusion of the nuclear remnants of related cells cannot wholly be excluded. Cervical, axillary, tracheobronchial, mesenteric, celiac and periaortic nodes and the lymphoid tissue of the ileum and the colon were studied. A comparison of the described atypical giant cell with the giant cell of untreated Hodgkin's disease can be made in figure 6.

Spleen.—Unlike that which is frequently encountered following severe roentgen irradiation, none of the spleens studied were smaller than normal. Thirty weighed over 300 Gm., the weight of the largest being 1,960 Gm. The external capsular surface was usually thickened and grayish white; in 4 instances it was adherent to the diaphragmatic surface—a phenomenon frequently noted in untreated leukemia. In most cases, on cut section the consistency was firmer and the

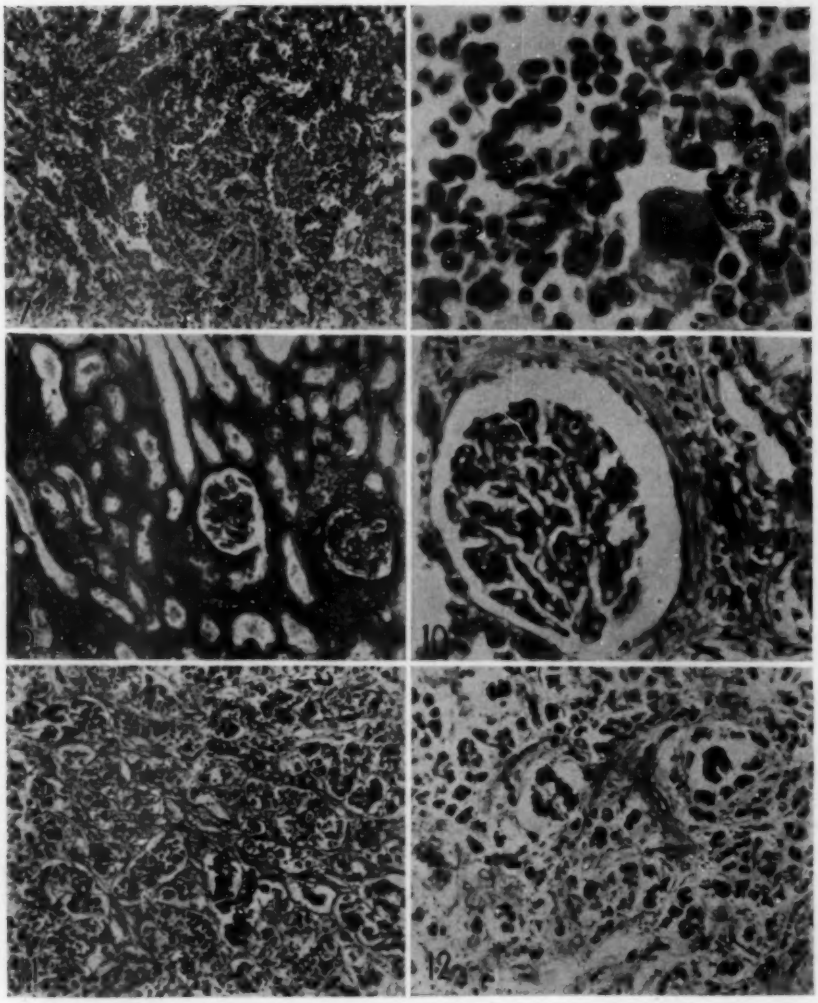

Fig. 7.—Spleen showing increased thickening of the basement membranes of sinusoids, with obliteration of some sinusoids and telangiectatic appearance of others. Notice the prominent, swollen endothelial cells lining these channels throughout and the focal area of eosinophilic fibrinous necrosis in the upper left hand corner. Hematoxylin and eosin; × 79.

Fig. 8.—Spleen. Note the large multinucleated giant cell and the abnormal mitotic figures therein and in surrounding cells. The nuclei are eccentrically placed, and the cytoplasm is dark eosinophilic. Hematoxylin and eosin; × 340.

Fig. 9.—Kidney of a 28 year old white man. Note the hyalinization and thickening of Bowman's capsule with slight thickening of the basement membrane of the glomerular tuft capillaries. Adjacent tubular structures show slight degenerative changes. Heidenhain-azocarmine stain; × 79.

Fig. 10.—Kidney of a 26 year old white man. Note thickening and hyaline change in Bowman's capsule with similar alterations in glomerular capillaries. Hematoxylin and eosin; × 380.

Fig. 11.—Pituitary gland showing moderate interacinous fibrosis with atrophy and necrosis of adjacent acidophilic and chromophobic cells. Hematoxylin and eosin stain; × 79.

Fig. 12.—Pancreas of a 28 year old white woman. Marked interacinous collagenous thickening with fibrous tissue formation and hyalinization are illustrated. The pancreatic cells show varying degrees of degenerative changes.

color grayer than normal, and prominent trabeculae traversed the splenic pulp. Microscopically, there was masking of the normal pattern by the malignant cellular invasion. Occasional extramedullary hemopoietic foci were also seen. This type of hemopoiesis was especially prominent in those cases in which the marrow had been severely damaged. Only a few degenerating lymphocytes were visible around the central arterioles of the malpighian corpuscles. However, those alterations which are more closely related to radiophosphorus therapy are an increase in fibrous tissue involving the trabeculae and the sinusoidal and arteriolar walls, focal fibrinous necrosis and hyalinization of these structures, and an increase in the number of multinucleated giant cells (fig. 8). The connective tissue increase was demonstrated in 28 cases, and in 16 of these, zones of acidophilic necrosis were also shown. The thickening of the basement membrane of the sinusoids had obliterated some of these vascular spaces. Other sinusoids were telangiectatic (fig. 7). The fibrotic and hyaline changes involving the media and the adventitia of the central arterioles and occasionally the larger vessels had produced partial narrowing and almost complete obliteration of the lumens. There were also moderate to striking hyperplasia and swelling of the endothelial cells with formation of vesicular, foamlike cells[7] in the intimal and medial coats. Similar changes were also observed in some of the blood vessels of the uterus (fig. 4), the marrow and the kidneys of patients in this series who were under 40 years of age. Occasionally there were whorls of fibrous tissue encircling portions of malpighian corpuscles. There was also a conspicuous deposit of hemosiderin in the red pulp.

Kidneys, Ureters and Bladder.—The renal changes observed after repeated intravenous injections of radioactive material can probably be attributed to the concentration that occurs in the nephron during the process of filtration and reabsorption. In addition, it has been previously determined[8] that renal tissue is in itself moderately responsive to radiation. In 32 of the cases included in this series there were vascular and tubular renal alterations. Since it is difficult to distinguish changes secondary to primary vascular disease and changes due to beta radiation effect, only those 15 cases in which the patient was below 40 years of age were considered sufficiently characteristic to be placed in the latter category. The most characteristic histologic changes were thickening and fibrosis of the renal capsule and hyalinization and thickening of Bowman's capsule (figs. 9 and 10) with only rare involvement of the basement membrane of the glomerular tuft. Also observed were hyperemia, swelling, vacuolation and desquamation of the epithelium of the tubules, especially in the convoluted tubules. The blood vessels were not severely damaged but occasionally showed some thickening of the intima. Infrequently, the interstitial tissue was condensed and hyalinized. In a few of the ureters examined, there was histologic evidence of fibrosis. The various stages of the reaction of the bladder to radioactive phosphorus were characterized by varying degrees of hyperemia, occasional edema, desquamation of epithelial cells and increase in hyalinized connective tissue involving the mucosa, submucosa and muscularis. There was no history of catheterization or cystoscopic procedures in the cases in which the aforementioned microscopic changes were observed. No specific effects were seen in the prostate and seminal vesicles.

Endocrine System.—Pituitary Gland: Histologic study of this endocrine organ revealed a slight to moderate increase of connective tissue distributed between the acini (fig. 11). In 1 case there was such marked fibrosis that compression

7. Sheehan, J. F.: Proc. Inst. Med. Chicago **15**:105, 1944.
8. Desjardins, A. U.: J. A. M. A. **83**:109, 1924.

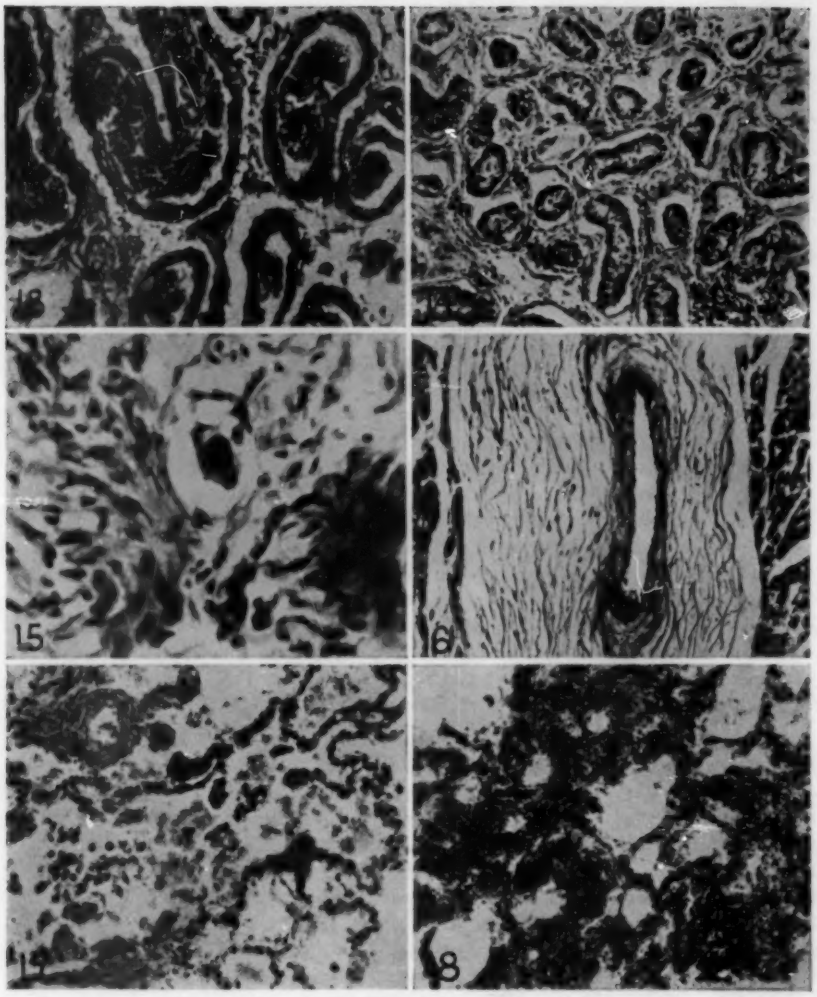

Fig. 13.—Testis of a 38 year old Negro man (no history of mumps or of testicular damage). Note the glassy hyalinization and thickening of the tunica propria and the basement membrane of each seminiferous tubule. No true spermatogenesis is visible. There is moderate edema of the interstitial tissues, with narrowing of the smaller vessels and almost complete disappearance of Leydig cells. Hematoxylin and eosin stain; × 79.

Fig. 14.—Testis of a 13 year old white boy (no previous history of mumps or of testicular damage). There is interstitial fibrosis with thickening of the tunica propria and the basement membrane of tubules. The latter have retracted from the basement membranes, and there is practically no spermatogenesis seen. Arterioles in the interstitial tissue show hyaline changes in the media. Hematoxylin and eosin stain; × 79.

Fig. 15.—Ovary of an 18 year old white woman (no history of mumps or of ovarian damage). The ovum shows degenerative changes involving the nucleus and the follicular epithelium. Hematoxylin and eosin stain; × 340.

Fig. 16.—Heart of a 28 year old white man (no history of pathologic changes of coronary arteries or rheumatic heart disease). Note fraying and separation of medial and adventitial fibers, with zones of hyalinization, in a branch of the coronary artery. Hematoxylin and eosin stain; × 79.

Fig. 17.—Lung showing degenerative metaplastic changes in the alveolar epithelium and thickening of the alveolar wall, with hyaline membrane formation and dilated alveoli. Note the hyaline changes in the medial coat of a small artery. Hematoxylin and eosin stain; × 79.

Fig. 18.—Hyaline alterations and slight fibrosis in pulmonary parenchyma, with narrowing and obliteration of some alveolar sacs. A small hyaline thrombus partially occupies the lumen of a vessel. Hematoxylin and eosin stain; × 79.

atrophy and necrosis of the adjacent eosinophilic and chromophobe cells were present. Other eosinophilic cells appeared swollen, the cytoplasm having an amorphous hyaline appearance. No associated clinical effects were observed.

Adrenal Glands: In 18 instances the capsules of both adrenal glands were markedly thickened by hyalinized connective tissue. The periadrenal arterioles showed similar hyaline degenerative changes in the medial coats. Because of the frequency of postmortem cellular changes occurring in these glands, it is difficult to distinguish minimal changes in the cortex. However, the greatest and most constant alteration was marked dissolution of cells and loss of cellular detail. These changes were observed in adrenal glands removed two to four hours after death.

Thyroid and Parathyroid Glands: No significant characteristic changes were observed in the small number of glands examined.

Pancreas: The same criterion of age was used in an effort to distinguish the fibrotic changes of age and associated vascular disease from alterations caused by radiophosphorus. Twenty-one pancreases (11 from patients below 40 years of age) showed moderate to marked interacinic and interlobular fibrosis with occasional hyaline changes in the collagen fibers (fig. 12). Three of these pancreases also revealed slight fibrosis of the islets. There were no duodenal ulcers and only minimal periductal reaction. There were edema of the collagen about the acini, vesiculation and desquamation of epithelial cells and occasional vacuolation and increased secretion of mucus by the ductal epithelium.

Reproductive Organs.—Testis: Of 16 testes from patients under 40 years of age, 11 showed varying degrees of destruction of the germinal epithelium. There were some tubules that showed almost complete disappearance of spermatocytes. Those that remained exhibited different stages of nuclear degeneration. In those testes showing the more severe changes, the only evidence of spermatogenesis that was observed consisted of fragmentary pyknotic nuclei. In practically all instances the seminiferous tubules showed thickening and varying degrees of hyalinization of the tunica propria and the basement membrane (fig. 13). Occasionally, amorphous groups of fused cells which resembled the Sertoli type were observed. There were also moderate to abundant edema of the interstitial tissue with diminution in the number of identifiable Leydig cells. In some cases the only significant abnormality was a mild degenerative change—for example, vacuolation of the cytoplasm of the tubular cells. A characteristic interstitial fibrosis, with practically no spermatogenesis in the tubules, was observed in still other testes (fig. 14).

Ovary: The most conspicuous alteration in ovarian tissue was the disappearance of the primary and graafian follicles. Those ova which remained either showed varying degrees of degeneration (fig. 15) or were replaced by a hyaline remnant. Those corpora lutea which were seen exhibited few significant changes. The interstitial tissue showed occasional areas of edema, lymphocytic infiltration or increased hyperemia, with telangiectatic vessels therein. These changes were observed in 6 of 7 ovaries studied, from patients who were under 40 years of age.

Heart.—Minimal changes were demonstrated in the myocardium and the coronary blood vessels of 14 of 21 patients under 40 years of age. These changes consisted of infrequent foci of granular and vacuolar degeneration of the myoplasm and the nuclei of individual cells. There were also occasional subepicardial and endocardial zones of hyalinized connective tissue. The vascular supply of the heart, especially the larger branches, showed moderate to severe fraying and separation of the medial and adventitial fibers, with small zones of hyalinization therein (fig. 16).

Lungs.—The pneumonitis caused by the beta radiation of P^{32} has been previously described as seen in experimental animals by Warren and Gates.[1b] The changes in the pulmonary tissue of 27 patients of the present series who were under 50 years of age were similar to the experimental findings and characteristic in 20 cases. The alterations ranged from moderate congestion, edema, lymphangiectasis, slight inflammatory cell infiltration and minimal degenerative metaplastic changes in bronchial and alveolar epithelium to well defined hyaline membrane formation (fig. 17), extreme thickening of alveolar walls, focal atelectasis, thickening of pulmonary vessels with associated swelling of collagen and hyaline degenerative changes therein. Moderate to severe fibrosis of pulmonary parenchyma was also observed (fig. 18). It is difficult to ascertain the relation of these changes to intercurrent infection, but the increase of connective tissue was not infrequently present in nonpneumonic, nonatelectatic and nonbronchiectatic pulmonary tissue. Occasionally various degrees of focal emphysema were associated with the hyaline membrane formation and the thickening of the wall.

Bone.—The significant features of the changes in bone can be summarized as follows: disappearance of osteoblasts and absence of osteocytes and lacunas. Occasionally the canaliculi were enlarged and irregular but reduced in number. Abnormal spaces sometimes appeared about the lamellas. Those osteocytes which remained showed pyknosis and vacuolation. In addition, it was difficult to distinguish any specific zone of demarcation between the normal and the irradiated bone. In reality, the reaction of adult bone to radioactive phosphorus was minimal when compared with the necrotic and more severe degenerative changes seen after heavy exposure to radium and roentgen radiation.

Skeletal Muscle.—Loss of cross striations, fragmentation of fibers, homogeneity of myoplasm and nuclear karyorrhexis were the characteristic microscopic changes seen in skeletal muscle fibers. Frequently Zenker's type of degeneration appeared. Occasionally, the less damaged fibers showed striking waviness of the myofibrils and loss of cross striations, which together accentuated the longitudinal fibrillation.

COMMENT

The increasing interest in temporarily radioactive isotopes, which are being used both as therapeutic agents and as tracers, necessitates a more detailed study of the microscopic changes noted in the body tissues as a whole. The fact that radioactive phosphorus is injected intravenously in most instances and that its 14.3 day half-life permits the tissues to be steadily irradiated for several weeks indicates that more diffuse cellular changes that are usually observed after local application of radium and roentgen radiation may be brought about. As can be determined by a study of the accompanying table, those tissues which utilize phosphorus rapidly and which also have a high phosphorus content, i. e., bone marrow, liver, spleen and lymph nodes, specifically take up higher concentrations of radioactive phosphorus than do normal tissues. Radioactive phosphorus is metabolized into nucleoproteins in the same manner as normal phosphorus (P^{31}). However, the rapidly proliferating cells take up much more radioactive phosphorus (P^{32}) than do normal, slow-growing cells. Therefore, proportionately higher concentrations of radioactivity are formed in those tissues preferentially involved in malignant lymphomas and primary polycythemia. Further

study of the table demonstrates an already obvious conclusion, i. e., that in almost every therapeutic application of radiation, normal tissues are affected as well as the intentionally irradiated focus of disease.[2] The profound sclerosing effect of radiophosphorus on the bone marrow has therefore led to variable hematologic complications in different patients, the peripheral and central response being independent of the dosage employed. According to Reinhard and his associates,[3] when two or more of the cellular elements of the marrow were depressed in the same patient, the formed elements of the peripheral blood showed changes in the following order: The leukocyte level decreased first, the thrombocyte level second and the erythrocyte level last.

The most prominent alterations in immature marrow cells occurred in the megakaryocytes. These cells were either degenerated or completely absent in most of the marrow sections studied. Thus, from the clinical and the pathologic observations, the graphic representation of decreasing cellular sensitivity to radioactive phosphorus should be modified so that it would differ slightly from that previously proposed by Dunlap and Warren [2b]—for example:

Leukocytes and Leukopoietic Tissue > Megakaryocytes and Megakaryocytic Tissue > Erythrocytes and Erythropoietic Tissue

The urinary excretion of radioactive phosphorus, after intravenous injection, varies from 5 to 25 per cent during the first four to six days in patients with leukemia and polycythemia vera.[9] However, approximately 25 to 50 per cent of the isotope is excreted by normal subjects within the same period. The difference in output is explained by Erf and Lawrence as being due to the quick fixation of radiophosphorus in the pathologic tissue and cells. This continued exposure of the renal parenchyma, with its extreme vascularity, to beta rays is probably the factor responsible for the thickening of Bowman's capsule and the associated tubular changes. Doub, Hartman and Bollinger [10] regarded the kidney as the organ most susceptible to roentgen irradiation as far as anatomic changes and loss of function were concerned. They pointed out that direct irradiation of the exposed kidney could result both clinically and experimentally in nephritis with hypertension and therefore advised avoiding irradiation of the renal areas in young persons. This same question may be raised in the use of radioactive phosphorus during the relatively long life expectancy of the younger patients with primary polycythemia. Although it is true that long clinical and hematologic remissions may be produced in primary polycythemia by one course of therapy, repeated treatment may, perhaps, lead to severe irreversible renal vascular disease and subsequent hyper-

9. Erf, L. A., and Lawrence, J. A.: J. Clin. Investigation **20**:567, 1941.
10. Doub, H. P.; Hartman, F. W., and Bollinger, A.: Radiology **8**:142, 1927.

tension. In fact, 11 of the 30 patients treated by Reinhard [8] for polycythemia vera had a systolic blood pressure of 150 mm. or more of mercury, and 8 of these 11 had a diastolic pressure of 100 mm. or more of mercury. Furthermore, of the abnormal physical findings, hypertension responded least favorably to radiophosphorus therapy. Unfortunately, the patients with malignant lymphomas do not live long enough for a proper evaluation of renal and blood pressure changes. As yet I have had no opportunity to study the kidneys of patients with polycythemia treated for prolonged periods with radiophosphorus.

It is pertinent to mention the theory of anoxemia of the marrow as a cause of polycythemia vera.[11] Certainly, the oftener observed arteriolar narrowing and marrow fibrosis after radioactive phosphorus therapy would not serve to increase the oxygen intake of the medullary cavity. A similar question might also be raised as to the relative importance of the pulmonary alveolar and arteriolar thickening. Since these changes are frequently associated with the secondary type of polycythemia, it is possible that they make a relative contribution to the continuation of the anoxemia, to the persistence of the polycythemia and perhaps eventually to the development of right ventricular hypertrophy. However, an important consideration to be weighed against the foregoing objections, is the almost complete prolonged hematologic and symptomatic remission seen following this new type of radiation treatment.

Finally, serious consideration must be given to the changes in the testes and the ovaries of patients who are in the reproductive period of life. Observation of these organs confirms the possibility that spermatogenesis and oogenesis may decrease or disappear, with development of sterility, in young persons given radiophosphorus.

SUMMARY

The beta emanations of radioactive phosphorus (P^{32}) produce characteristic changes in many body tissues, alterations which vary in intensity but are similar to those produced by other types of radiation.

The lesions observed are the resultant of several diverse cellular, intercellular and vascular changes. Prominent specific features are varying degrees of cellular death, abnormal mitoses producing giant irregular nuclei, fibrosis, hyalinization of collagen and a vascular alteration characterized by thickening and hyalinization of small blood vessels.

There is selective localization of larger quantities of radioactive phosphorus and greater injury in the tissues most often involved by the neoplastic cells of malignant lymphomatoses and myelomatoses, i. e., marrow, liver, spleen and lymph nodes. Changes were also demonstrated in lungs, kidneys, gastrointestinal tract, ovaries and testes.

11. Reznikoff, P.; Foot, N. C., and Bethea, J. M.: Am. J. M. Sc. **189**:753, 1935.

BRAIN REPAIR

II. Role of the Lipids of the Brain in the Genesis of Gliosis

SAMUEL PENDLETON HICKS, M.D.
Chief Pathologist, United States Naval Medical School, National Naval Medical Center
BETHESDA, MD.

THE CELLULAR processes which lead to repair after injury of the brain have been studied by a number of workers. The Macklins,[1] Russell [2] and Carmichael [3] studied the function of the brain phagocytes, variously designated macrophages, gitter cells and microglia, and found them to be the major cellular participants in the acute reaction following injury by puncture. These phagocytes, which have been shown by Dunning and Furth [4] to be members of the histiocyte-monocyte or so-called reticuloendothelial system, ingest cellular debris and erythrocytes in foci of necrosis or in the wounds caused by mechanical punctures of the brain. This process is most active during the first week, but the cells persist in wound tracks or other foci of injury for as long as several months to almost a year in some instances. That these same cells in the spleen exhibit strong proteolytic enzymatic activity has been demonstrated by Hicks and Opie,[5] but in the brain the observation that the phagocytes persist at the site of injury, laden with lipids, for weeks and months, suggests that they are slow in digesting myelin and other fatty substances derived from brain tissue. During the acute reactive phase of the first week, there is also a proliferation of fibroblasts, the degree of which is determined by the amount of damage to the fibrous tissue accompanying blood vessels or the fibrous tissue of the meninges. Penfield and others [6] showed that the amount of cicatrix forming in a puncture wound of the brain was proportional to the amount of damaged tissue left behind by the needle. A solid needle introduced meningeal

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The opinions and views set forth are those of the writer and are not to be cconsidered as reflecting the policies of the Navy Department.

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2. Russell, D.: Am. J. Path. **5**:451, 1929.
3. Carmichael, E. A.: J. Neurol. & Psychiat. **9**:209, 1929.
4. Dunning, H. S., and Furth, J.: Am. J. Path. **11**:895, 1935.
5. Hicks, S. P., and Opie, E. L.: Am. J. Path. **18**:333, 1942.
6. Penfield, W., and del Rio-Hortega, P.: Bull. Johns Hopkins Hosp. **41**: 278, 1927. Penfield, W., and Buckley, R. C.: Arch. Neurol. & Psychiat. **20**:1, 1928. Penfield, W.: Surg., Gynec. & Obst. **39**:803, 1924.

connective tissue into the wound and the result was that in the wound track a fibrous core developed which often led to cicatricial contracture of the surrounding brain and meninges later on. A hollow needle, however, by removing a core of damaged tissue, left almost no injured tissue behind and produced little fibrous tissue damage and reaction. Such an injury healed with minimal scarring and contracture, a matter of importance in avoiding possible postoperative epilepsy. Gliosis, which is a proliferation of fibrillary astrocytes and a laying down of their fibrils, appears to be a secondary reaction in brain repair.[7] Beginning changes in the astrocytes which lead to later proliferation have been observed around some experimental puncture wounds a few days after injury, but gliosis when it develops does not become conspicuous until about the third week, and the laying down of fibrils progresses for many weeks. It acts as a secondary repair mechanism in the brain. Around simple puncture wounds it may be almost absent or may develop at the margin of the track if that tissue has been injured. Carmichael and associates[8] investigated the histologic sequence in the development of abscesses of the brain, devoting considerable study to the development of gliosis. They found that the primary response was that of the phagocytes and the proliferation of fibrous tissue around the injury. Astrocytes reacted secondarily around the primary process, producing a radially arranged mat of glial fibrils just outside the fibrous tissue wall and forming a less concentrated zone of loosely arranged astrocytes beyond the mat. This same pattern of reaction may occur around large hemorrhages and some other lesions. The stimulus to gliosis appears to be, therefore, a sublethal injury of the astrocytes, for their proliferation always occurs in a zone around a primary region of destruction or in a region where damage falls short of diffuse necrosis, as in anoxic, toxic, hypoglycemic and demyelinizing processes.[9] The sublethal injury may possibly be due to bacterial toxins diffusing outward from the primary site of an abscess, or to the relative anoxia which so often is associated with hemorrhage. A further example is seen in the astrocytes which in some cases of hyperinsulinism[10] proliferate in regions of the forebrain, which suffer from hypoglycemia sufficiently that many of the nerve cells[11] are destroyed but not so much that there is wholesale necrosis of brain tissue.

The sequence of events in repair of the brain after several general types of injury is summarized in the table.

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7. Linell, E. A.: Arch. Neurol. & Psychiat. **22**:926, 1929. Rand, C. W., and Courville, C. B.: ibid. **27**:605, 1932. Cone, W.: ibid. **20**:34, 1928.
8. Carmichael, F. A., and others: Arch. Neurol. & Psychiat. **42**:1001, 1939.
9. Ferraro, A.: Arch. Neurol. & Psychiat. **52**:443, 1944.
10. Hoff, E. C.: Medicine **24**:161, 1945.
11. Chesler, A., and Himwich, E. E.: Arch. Neurol. & Psychiat. **52**:114, 1944.

	Simple Puncture Wound	Hemorrhage with Anoxia	Abscess	Injury Without Diffuse Necrosis, as in Anoxic, Hypoglycemic, Toxic and Demyelinizing Processes
1st and 2d wk.....	Brain phagocytes ingest blood cells and elements of destroyed brain tissue. Fibroblasts and few capillaries grow from injured meningeal vascular sources into wound track. Phagocytosis reaches maximum during first week.	Brain phagocytes ingest blood cells and elements of destroyed brain tissue. Fibroblasts and few capillaries from injured vessels proliferate around hemorrhage, gradually walling it off	Reaction same as in hemorrhage, but more intensive, and polymorphonuclear cells from blood also accumulate in abscess in response to certain organisms, e. g., staphylococci	Brain phagocytes ingest destroyed myelin, dead neurons and processes. Proliferation of fibrous and vascular tissue is absent unless these elements are injured; then their reaction is usually slight
3d and 4th wk.....	Phagocytes persist; they contain lipids and blood pigments. Fibrosis attains maximum about 3d week. Gliosis (proliferation of astrocytes and their fibers) at margin of wound track is absent unless the surrounding tissue is injured	Phagocytes continue activity if lesion is large. Fibrous wall increases about hemorrhage. Proliferating astrocytes have become conspicuous around the fibrous wall, with radially arranged fibrils	Reaction as in hemorrhage, but injury to and proliferation of fibrous tissue are usually considerably greater, with formation of a thick wall. Gliosis has become conspicuous	Phagocytosis subsides unless there is continuing injury as in some demyelinizing diseases. Proliferation of astrocytes (gliosis) is progressing
After 4th wk.....	Phagocytes containing lipids and blood pigment persist in wound track for months but diminish progressively. Fibrous tissue diminishes. Gliosis is not conspicuous unless surrounding tissue is injured. Fibrosis is the predominant feature of the end result	Phagocytes persist for weeks or months, depending on presence of persisting hemorrhage. Fibrous and glial wall about lesion with or without cyst formation is end result	Phagocytes persist for weeks or months, depending on balance between healing and necrosis. Fibrous and glial walls become prominent around abscess. End result is healing with fibrosis and gliosis, with or without cyst formation	End result is replacement of dead or degenerated tissue with fibrillary astrocytes: gliosis. Fibrosis is usually inconspicuous

As seen from the table, the gliosis developing in response to injury of the brain is a secodary reaction that is specific to the nervous system. The initial inflammatory reaction, characterized by a cellular exudate of phagocytes and followed by fibrosis and by granulation tissue in which fibroblasts predominate, is a nonspecific reaction seen in all tissues of the body.

The question arises whether gliosis is also stimulated directly by the injurious agent, or whether the stimulus arises secondarily from the liberation of lipoid substances of destroyed nerve cells and their myelin sheaths. This problem is particularly interesting in regard to the demyelinizing diseases of the nervous system. Bender [12] sought to throw some light on the problem by introducing several extracts of brain into the brains of dogs and studying the effect on repair after four weeks. Her results suggested that gliosis was stimulated to some extent by these substances, but the exact nature of the extracts used was not specified.

The following study was therefore undertaken to determine whether certain lipids of the brain when introduced by puncture into the brains of white mice would stimulate gliosis over and beyond that which is sometimes observed in simple puncture wounds. The lipids employed were sphyngomyelin, lecithin, cholesterol and whole brain lipids. The wounds were compared histologically with simple puncture wounds, as controls, with the reaction to a foreign body (bone) and with the reaction to bacterial infection (avian tubercle bacilli).

CYTOLOGIC EXPERIMENTS

Young adult male and female white mice between the ages of 6 weeks and 3 months were used in all experiments. They were obtained from the stocks used for routine laboratory purposes at the Naval Medical School and the National Institute of Health. Under ether anesthesia and aseptic conditions, punctures of the brain were made with hypodermic needles to a measured depth of 4 mm. To insure uniform depth of the punctures a guard was used on each needle, set at 4 mm. The needle was introduced through the shaved skin and the skull into the middle of each cerebral hemisphere, puncturing the meninges, the cerebrum, the interbrain and often the lateral ventricle. Ventricular hemorrhage was never conspicuous. Suspensions of the lipids, avian tubercle bacilli and tiny bone fragments were introduced by the same technic. The suspensions of the lipids and the bone particles were made up in distilled water; those of the bacilli, in isotonic solution of sodium chloride. One one-hundredth of a cubic centimeter of suspension was injected when the needle had been introduced into the brain. The animals were killed rapidly with ether at varying intervals up to two hundred and seventy days after injury. The brain was removed and fixed at once in 10 per cent neutral solution of formaldehyde, U. S. P., or Zenker's solution with 5 per cent acetic acid and was stained with hematoxylin and eosin and Mallory's phosphotungstic acid–hematoxylin to emphasize astrocytic glial fibrils. An autopsy was done on each animal. No

12. Bender, L.: Am. J. Path. **1**:657 and 667, 1925.

significant lesions were noted except those at the sites of brain puncture and lesions in the tuberculous animals, which will be described. The lungs, the livers and the spleens of tuberculous animals were examined microscopically after treatment with acid-fast stains as well as with hematoxylin-eosin and phosphotungstic acid–hematoxylin.

Simple Puncture of the Brain.—Twenty-six animals were divided into three series, and each cerebral hemisphere was punctured as described in the foregoing paragraph, three types of hypodermic needles being used: a standard hollow no. 23 needle, a standard no. 20 needle and an occluded no. 20 needle. Microscopically, the lesions produced by the three types of needles were remarkably uniform, and no noteworthy difference could be distinguished between the series, because each left behind the same amount of debris, and the lumen present in a needle in the absence of an occluding stylet was too small for a core of tissue to be removed in the manner employed by neurosurgeons. In a series of 14 animals the brain was punctured with the standard hollow no. 23 needle. One was killed four hours after injury and the rest on the second, third, fourth, sixth, eighth, eleventh, twelfth, fourteenth, seventeenth, twentieth, thirty-first, forty-second and sixty-second days after injury, respectively. In a second series of 6 animals the brain was punctured with the standard hollow no. 20 needle, and these mice were killed two, five, twelve, fifty, sixty and eighty days after injury. In a third series of 7 animals the brain was punctured with the occluded no. 20 needle, and 1 was killed on the fifth, 4 on the ninth, sixteenth, twenty-first and thirty-first days and 2 animals on the two hundred and seventieth day after injury.

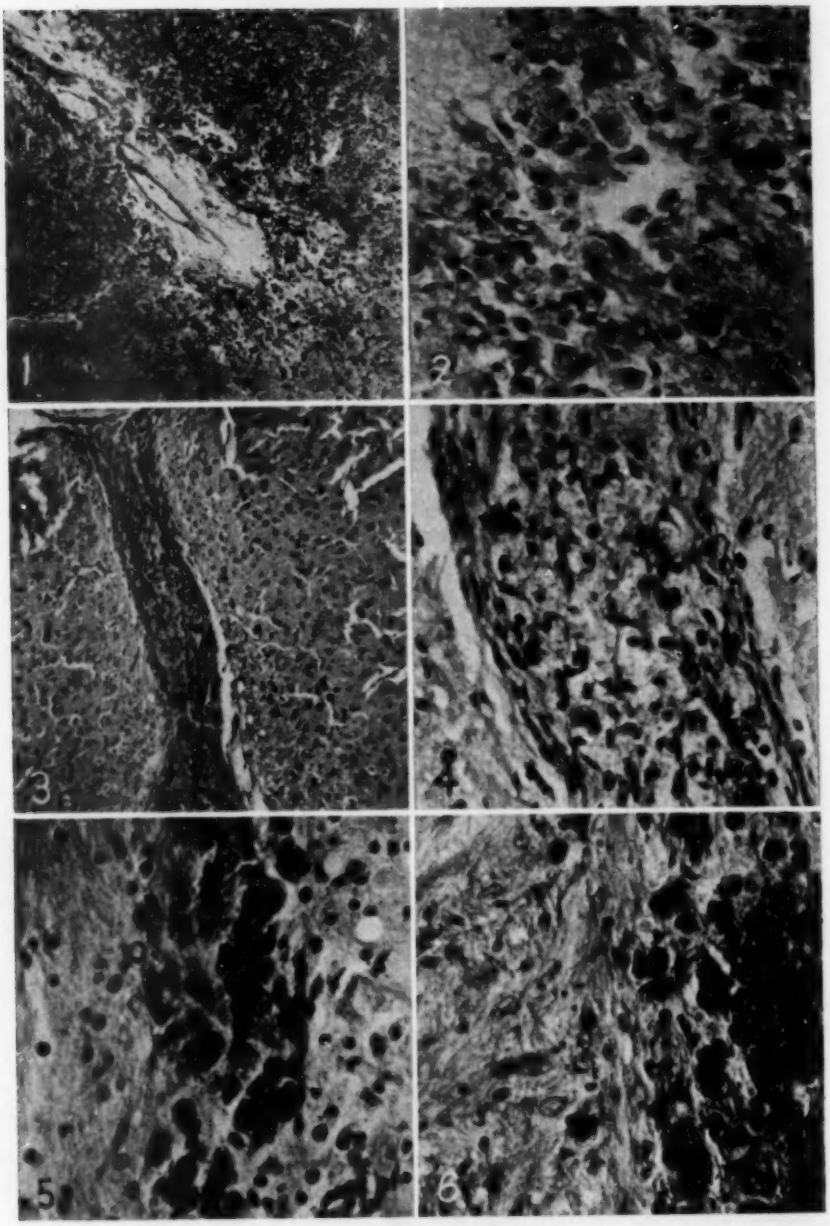
Puncture with Introduction of Sphyngomyelin.[13]—One one-hundreth of a cubic centimeter of a freshly prepared sterile 2 per cent suspension of sphyngomyelin was introduced by puncture with a no. 23 needle into each cerebral hemisphere of 4 mice. One was killed twelve days after injury, another on the twenty-second day and two on the two hundred and seventieth day after injury.

Puncture with Introduction of Lecithin.[13]—One one-hundreth of a cubic centimeter of a freshly prepared sterile 5 per cent suspension of lecithin was introduced by puncture with a no. 23 needle into each cerebral hemisphere of 4 mice. They were killed three, twelve, twenty-four and forty-four days after injury.

Puncture with Introduction of Cholesterol.—One one-hundredth of a cubic centimeter of a sterile approximately 2 per cent suspension of pure crystalline cholesterol was introduced by puncture with a no. 20 needle into each cerebral hemisphere of 3 mice. They were killed on the third, sixteenth and forty-fourth day after injury.

Puncture with Introduction of Whole Brain Lipids.—One one-hundredth of a cubic centimeter of a sterile suspension of whole brain lipid was introduced by puncture with a no. 23 needle into each cerebral hemisphere of 5 mice, which were killed on the third, twelfth, twentieth, thirty-second and forty-first days after injury. The suspension was prepared from the aseptically removed brain of a normal white mouse, which weighed 0.4 Gm. This was macerated and extracted for thirty minutes in the cold with 10 cc. each of absolute ethyl alcohol and ether, and filtered. The filtrate was evaporated at 40 C. with an air jet, and the residuum

13. These phosphatides were prepared at the Naval Medical School as a by-product of Kahn test antigen. The terms "sphyngomyelin" and "lecithin" represent specific closely related groups of substances rather than single chemical entities (Page, I. H.: Chemistry of the Brain, Springfield, Ill., Charles C Thomas, Publisher, 1938.)



FIGURES 1 TO 6

(See legends on opposite page)

suspended in 1 cc. of distilled water. On the assumption that fresh brain is roughly 10 per cent lipids, the suspension was estimated to be about 4 per cent lipid.

Puncture with Introduction of Foreign Body (Bone Particles).—One one-hundredth of a cubic centimeter of a sterile suspension of bone particles was introduced with a no. 23 needle into each cerebral hemisphere of 5 mice. They were killed three, twelve, twenty, thirty-two and forty days after injury. The suspension of bone particles was made by grinding aseptically a piece of the skull bone of a normal white mouse in a mortar and suspending it in sterile distilled water. The resulting suspension was milky, and the particles passed through the no. 23 needle.

Puncture with Introduction of Avian Tubercle Bacilli.—One one-hundredth of a cubic centimeter of a suspension of avian tubercle bacilli was introduced with a no. 23 needle into each cerebral hemisphere of 4 mice. They were killed twenty hours, five days, twelve days and forty-eight days after injury. The suspension was prepared under aseptic conditions and made up in isotonic solution of sodium chloride from a six week old culture of virulent avian tubercle bacilli, grown at the National Institute of Health (Phipp's avian strain), and contained 1 mg. of bacilli per cubic centimeter.

RESULTS

Simple Puncture.—The histologic sections of the series of wounds caused by simple puncture of the brain showed virtually the same cytologic sequence of events described by others. After the first forty-eight hours, when hemorrhage with some fibrin filled the track formed by the needle, an exudate of phagocytes accumulated at the margins of the track and ingested the red blood cells and cellular debris. Polymorphonuclear leukocytes were extremely rare. This phagocytic reaction attained a maximum by the end of the first week. (See figures 1 and 2.) The chief cellular participant was the mononuclear phagocyte, which most observers believe is derived from the microglia of the

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Explanation of Figures 1 to 6

Fig. 1.—Wound track six days after simple puncture of the brain; × 81; phosphotungstic acid–hematoxylin. The track contains blood with some fibrin, and in its margin there are large numbers of phagocytes containing lipid and a few hemosiderin granules. The surrounding tissue is intact and contains some phagocytes en route to the wound track.

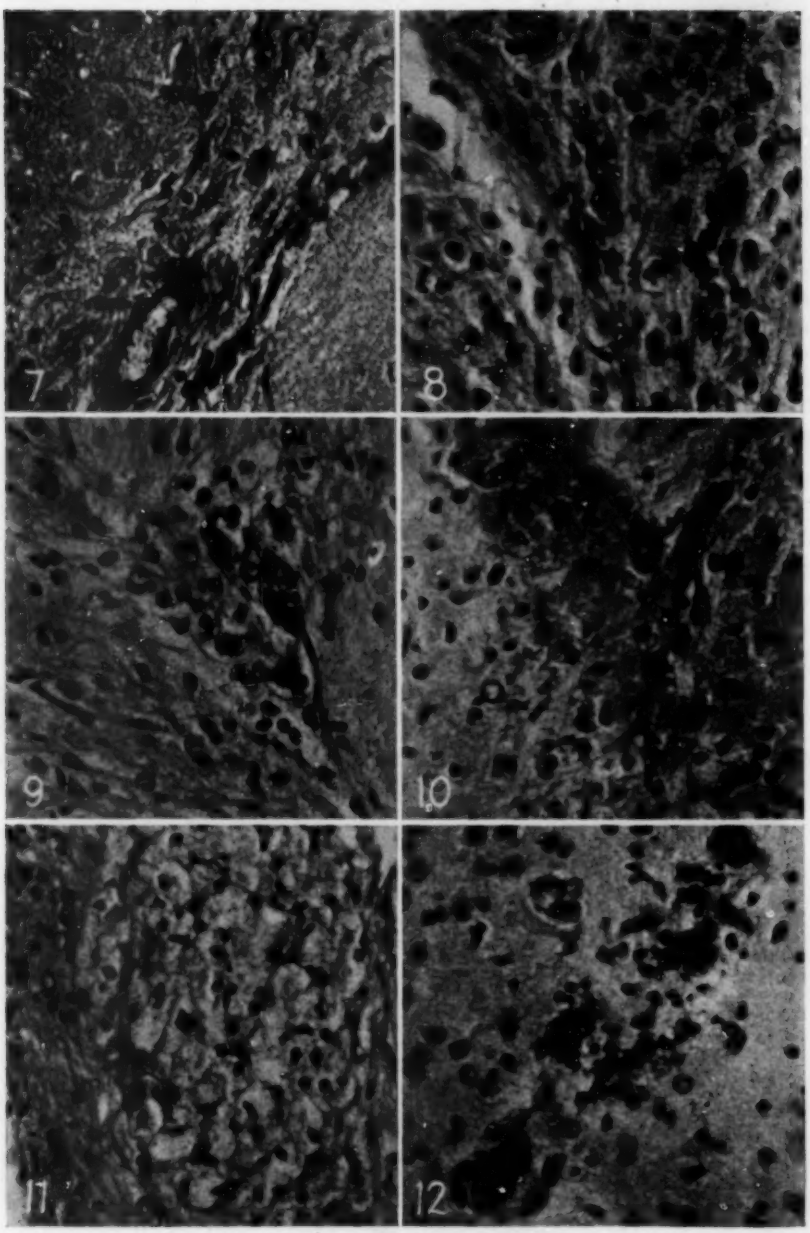
Fig. 2.—Lower end of the track seen in figure 1, × 324, to show phagocytes.

Fig. 3.—Wound track twenty-one days after simple puncture; × 81; hematoxylin and eosin. A loose core of fibrous tissue extending into the track from the meninges is seen. The track is packed with phagocytes containing lipids and hemosiderin. These two substances are irregularly distributed in the cells.

Fig. 4.—The midportion of the track seen in figure 3; × 324. No astrocytic glial reaction is seen around the wound track.

Fig. 5.—Wound track thirty days after simple puncture; × 324; hematoxylin and eosin. The track is composed of phagocytes and a loose core of connective tissue. Sections cut to show different parts of the track showed the usual irregular distribution of hemosiderin and lipids in the phagocytes.

Fig. 6.—Wound forty-two days after simple puncture, × 324; phosphotungstic acid–hematoxylin. The appearance is virtually the same as that at thirty days. Hemosiderin is prominent in this portion of the track, but lipids constituted the major part of the phagocytosed material in other portions. No gliosis is evident.



FIGURES 7 TO 12

(See legends on opposite page)

brain and the monocytes of the blood stream. That these cells multiplied at the site of injury was evidenced by occasional nitotic figures in them, a fact recorded also by others. The possibility that they were formed in situ from the connective tissue about the blood vessels or from lymphocytes [14] was not evident from these studies. By the end of the first week the cytoplasm of these phagocytes became heavily laden with lipids and blood pigment, some cells containing more of one substance than the other; with their included material some of them persisted in the wound tracks for as long as two hundred and seventy days. However, after the first few weeks there was progressive diminution of the number of such cells present.

Fibrous tissue proliferation developed *pari passu* with the acute phagocytic response. At first, the connective tissue fibers were found in association with the ingrowth of a few capillaries, which began within the first week. Neither this capillary ingrowth nor the pericapillary fibroblastic response was conspicuous. There was, however, a conspicuous ingrowth of fibrous tissue from the injured meninges overlying the lesion from the first few days after injury. By the second and third week it was sufficient to form a loose, irregular core of fibrous tissue, which occupied the wound track and was filled with persisting phagocytes. This picture continued through the thirtieth, forty-second, fiftieth, sixtieth and eightieth days, diminishing gradually. (See figures 3 to 7.)

14. Kulouch, F., Jr.: Am. J. Path. **15**:413, 1939.

EXPLANATION OF FIGURES 7 TO 12

Fig. 7.—Wound two hundred and seventy days after simple puncture of the brain; × 324, phosphotungstic acid–hematoxylin. It consists of a few fibrous tissue strands and scattered phagocytes containing hemosiderin and little lipid. No gliosis is present.

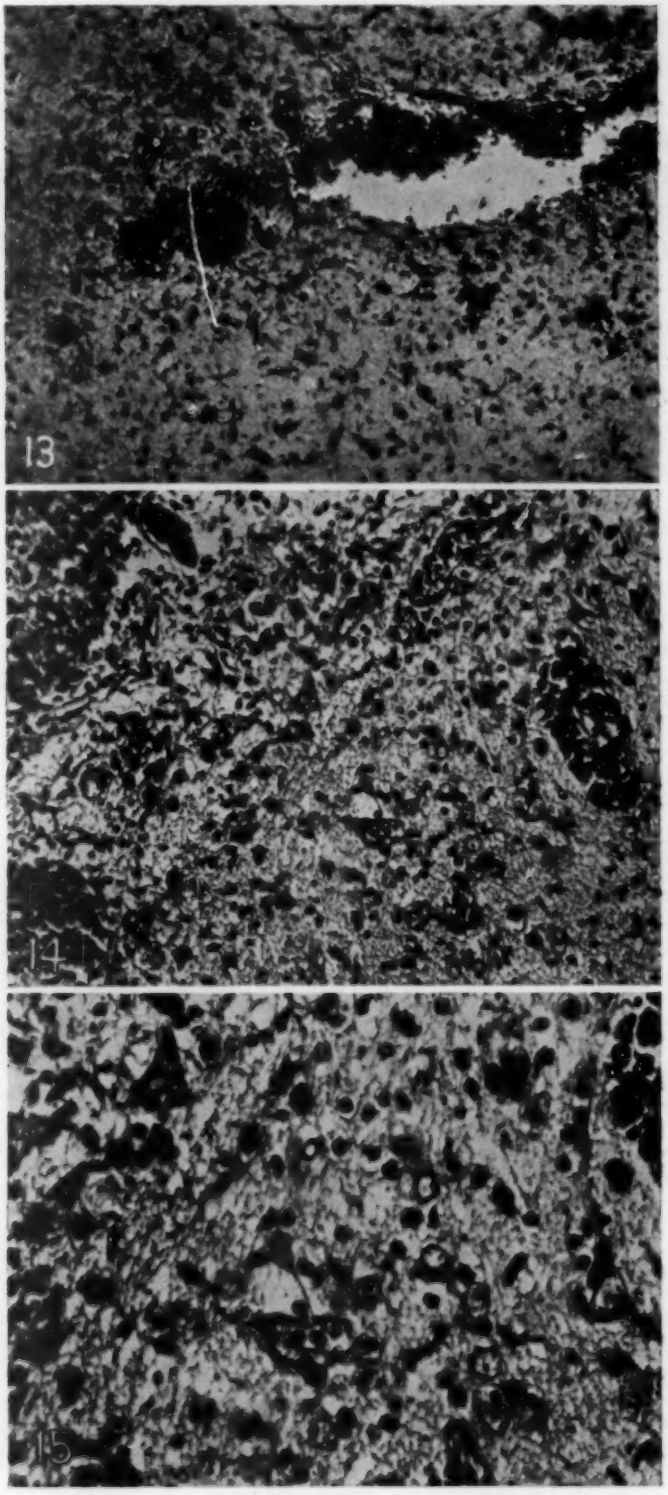
Fig. 8.—Wound two hundred and seventy days after puncture with introduction of sphingomyelin; × 324; hematoxylin and eosin. The appearance is virtually the same as that of the wound shown in figure 7. No gliosis is present.

Fig. 9.—Wound forty-four days after puncture with introduction of cholesterol; × 324; hematoxylin and eosin. It is shown in cross section and is composed of a few strands of fibrous tissue and phagocytes containing lipid and hemosiderin granules. No gliosis is evident.

Fig. 10.—Wound thirty-two days after puncture with introduction of whole brain lipids; × 324; hematoxylin and eosin. The track consists of strands of loose fibrous tissue and phagocytes containing lipid and hemosiderin granules. No gliosis is evident.

Fig. 11.—Wound forty-four days after puncture with introduction of lecithin; × 324; hematoxylin and eosin. The track is composed of phagocytes containing lipids and hemosiderin granules and a loose meshwork of connective tissue. There is no gliosis.

Fig. 12.—Wound thirty-four days after puncture with introduction of bone particles; × 324; hematoxylin and eosin. This section shows the deep end of the track, where fibrosis and reaction to tissue injury are minimal. The bone particles are exciting almost no reaction. There is no gliosis.



FIGURES 13 TO 15

(See legends on opposite page)

By the two hundred and seventieth day following the injury produced by the puncture, the fibrous track had narrowed to a few strands of connective tissue intermingled with a few pigmented phagocytes. This blood pigment was rarely seen outside the phagocytes, which contained little lipid at this stage.

Throughout the entire course of the experiment, the uninjured brain tissue seen in sections around the wound tract showed little or no response to the injury. There was almost no proliferation of astrocytes. Glial fibrils could be seen only extremely rarely in some wound tracks after the third week, and were longitudinally disposed at the margins. They were so infrequent as hardly to justify the use of the term gliosis. The narrowing of the track was attributed to the gradual disappearance of phagocytes and the shrinking and disappearance of fibrous tissue. This was reflected by some contracture of the surrounding brain toward the track seen after the sixth week.

Puncture with Introduction of Sphingomyelin, Lecithin, Cholesterol or Whole Brain Lipids.—The introduction of the various lipids, sphingomyelin, lecithin, cholesterol and whole brain lipids, did not alter the response from that seen with simple puncture. (See figures 8, 9, 10 and 11.) Again, the sequence of events was destruction of brain tissue with hemorrhage, phagocytosis and fibrosis. There was no evidence of cholesterol crystals or of the other lipids in the wound tracks. In none of the sections was there evidence of astrocyte proliferation with formation of glial fibrils. The lipids introduced did not, therefore, stimulate gliosis in the tissue surrounding the wound tracks and seemed to be without effect on brain tissue.

Puncture with Introduction of Bone Particles.—The bone particles deposited in the wound track after puncture did not alter the sequence of events. They remained in place without exciting a reaction greater than that seen in the control puncture wounds; the phagocytic and

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EXPLANATION OF FIGURES 13 TO 15

Fig. 13.—Wound forty-eight days after puncture with introduction of living avian tubercle bacilli; × 100; phosphotungstic acid–hematoxylin. The wound track is filled with phagocytes containing lipid, blood pigment and many acid-fast bacilli. At the margins of the track there is some granulation tissue, toward which there are somewhat radially arranged astrocyte fibrils. In the zone around the wound track there are scattered plump fibrillary astrocytes. This zone shows little other change, the usual structure with some nerve cells being still evident.

Fig. 14.—A portion of the track shown in figure 13; × 200. The phagocytes in the wound track are seen to the left, and loose granulation tissue appears to the right of them, merging into the surrounding brain, which shows considerable proliferation of astrocytes and their fibrils. A small blood vessel to the right, in this zone, shows perivascular lymphoid cells.

Fig. 15.—A portion of the section shown in figure 14, × 400, to show astrocyte proliferation.

fibroblastic response progressed and began to subside during the nearly six weeks of observation. Figure 12 shows several particles at the edge of the lower end of one of the tracks. There is a minimum of cellular response to these particles. No gliosis was stimulated.

Puncture with Introduction of Avian Tubercle Bacilli.—Avian tubercle bacilli altered the response to injury considerably when introduced into the brain by puncture. (According to Rich,[15] white mice are moderately susceptible to avian tuberculosis.) In the animal killed forty-eight days after puncture the wound tracks were filled with phagocytes containing lipids, blood pigment and acid-fast bacilli in large numbers. There were fibroblasts scattered in the track, and at the margins they were associated with a moderate number of new capillaries. Just at the outer edge of this zone there were fairly numerous astrocytes with proliferated fibrils arranged in somewhat radial fashion toward the wound track. A number of scattered plump fibrillary astrocytes were seen in a broad zone around the wound track where tissue destruction was minimal as evidenced by persistence of some nerve cells and most normal structures. (See figures 13, 14 and 15.) This gliosis was attributed to toxins diffusing into this zone, sufficient to cause sublethal injury but little necrosis. The animals killed twenty hours, five days and twelve days after the introduction of tubercle bacilli showed wound tracks comparable to those of the controls, but the phagocytes also contained acid-fast bacilli. The five day animal showed a few metastatic tuberculous foci in the liver and the spleen.

CONCLUSIONS

The experiments described, coupled with common observation, point to direct injury of astrocytes as the stimulus necessary for the production of gliosis. Astrocytes proliferate and lay down fibrils in regions of the brain that suffer damage from toxins and injurious processes sufficient to inflict minimal damage and kill a few nerve cells and their processes. The lipids liberated by destruction of brain tissue do not seem to stimulate gliosis. Were the lipids a stimulating factor, glial proliferation at the margin of the tracks ought to have been evident in both the lipid and the simple puncture experiments. Proliferation of astrocytes is a secondary healing mechanism. Astrocytes, unlike fibroblasts in association with capillaries, cannot organize dead tissue or hemorrhage. The fibroblasts may replace and fill up a defect caused by complete necrosis, as in an abscess, but the astrocytes cannot. Thus they are seen at the edges of areas of complete necrosis; only in places where necrosis is incomplete do they serve as replacement.

15. Rich, A. R.: Pathogenesis of Tuberculosis, Springfield, Ill., Charles C Thomas, Publisher, 1944, chap. 5, pp. 120 and 124.

SUMMARY

Studies were undertaken to determine whether (*a*) the setting free of lipids in foci of destruction of the brain or (*b*) direct injury of astrocytes is the stimulating factor in the production of gliosis.

The histologic sequence of events following simple punctures of the brains of white mice was compared with that seen after punctures in which sphingomyelin, lecithin, cholesterol and whole brain lipids were introduced into the wound tracks. A similar comparison was made with punctures into which bone particles and avian tubercle bacilli were introduced.

The histologic sequences following simple puncture of the brain, puncture with introduction of the brain lipids, and puncture with introduction of bone particles were the same: initial phagocytosis of damaged tissue and hemorrhage, followed by fibrosis. The lipids and the bone particles did not alter the response seen after simple puncture. Gliosis was almost entirely absent.

The sequence following puncture of the brain with introduction of avian tubercle bacilli was altered from that seen in the simple puncture and puncture with introduction of the brain lipids. Not only were phagocytosis and fibrosis evident but also, at the margins and in the zone around the wound tracks, proliferation of astrocytes and their fibrils.

It was concluded that proliferation of astrocytes and their fibrils is dependent on direct injury of the astrocytes and is not due to any stimulating action of the lipids liberated in the destruction of brain tissue.

EXPERIMENTAL STUDY OF PURPURIC MENINGOCOCCEMIA IN RELATION TO THE SHWARTZMAN PHENOMENON

With Discussion of Meningococcic Purpura, the Waterhouse-Friderichsen Syndrome and Bilateral Renal Cortical Necrosis

B. BLACK-SCHAFFER, M.D.

T. G. HIEBERT

AND

G. P. KERBY, M.D.

DURHAM, N. C.

MENINGOCOCCIC bacteremia characterized by cutaneous purpura has attracted much attention by virtue of its frequently fulminating course, its striking cutaneous manifestation, its unpredictable selectivity and finally the occasional involvement of the adrenal glands by necrosis and hemorrhage.

Despite excellent descriptions [1] of these lesions, their genesis remains a matter of hypothesis. In 1904 Langmead [2] suggested that the lesions might be due to the blood-borne toxin of an unknown disease agent. MacLagen and Cooke [3] proposed that the meningococcus selectively affects tissues of ectodermal origin. Impressed by the presence of eosinophilic leukocytes in various viscera, Glanzmann [4] discussed the possibility of an anaphylactoid reaction to the toxins of a septic state. Sachs [5] expressed the belief that the simultaneous presence of adrenal hypoplasia and the thymicolymphatic state exerted an important influence on the course of the disease. Shwartzman [6] proposed that the disease might be a resultant of the Shwartzman phenomenon. György and co-workers [7] suggested as a remote possibility that an acute bacterial septic state was superimposed on a nutritional deficiency. Jones,[8] from his study of 5 nonfatal cases, concluded that an allergic constitution might

From the Department of Pathology, Duke University School of Medicine.

1. (*a*) Banks, H. L., and McCartney, J. E.: Lancet **1**:219, 1942; (*b*) **1**:771, 1943. (*c*) Herbut, P. A., and Manges, W. E.: Arch. Path. **36**:413, 1943.

2. Langmead, F.: Lancet **1**:1496, 1904.

3. MacLagen, P. W., and Cooke, W. E.: Lancet **2**:1054, 1916.

4. Glanzmann, E.: Jahrb. f. Kinderh. **138**:49, 1933.

5. Sachs, M. L.: Ann. Int. Med. **10**:1105, 1937.

6. Shwartzman, G.: Phenomenon of Local Tissue Reactivity, New York, Paul B. Hoeber, Inc., 1937.

7. György, P.; Goldblatt, H.; Miller, F. R., and Fulton, R. P.: J. Exper. Med. **66**:579, 1937.

8. Jones, B. B.: Virginia M. Monthly **72**:32, 1945.

be a predisposing factor in the genesis of the disease. None of the theories mentioned is supported by adequate experimental or clinical evidence.

In 1928 Shwartzman [9] demonstrated a necropurpurogenic factor in meningococci. This was accomplished with bacterial culture filtrates inoculated intradermally (preparatory) and followed, optimally, twenty-four hours later with an intravenous (reacting) injection of the same filtrate. Within three to four hours after the reacting injection (also called the provocative inoculation) the skin at the site of preparation became hemorrhagic and then necrotic.

Because it was believed that a demonstrable relationship might exist between the lesions of meningococcic purpura and the Shwartzman phenomenon, the following three experiments were undertaken.

EXPERIMENT 1

Method.—Meningococci (strain P_3), isolated from the blood of a patient who had developed widespread cutaneous purpura, were grown on dextrose-starch-agar slants for twenty-four hours. The growth on each slant was washed off with 1 cc. of sterile isotonic solution of sodium chloride. The washings were centrifuged until the supernatant fluid was clear, whereupon it was decanted and sterilized by heating for fifteen minutes at 60 C. This material is hereafter referred to as "sterile supernatant α." The remaining meningococcic suspension was made up to its original volume or more by the addition of isotonic solution of sodium chloride and centrifuged a second time until the supernatant fluid was clear. The second decanting was followed by a second dilution with isotonic solution of sodium chloride centrifugation and further decanting. The organisms were resuspended for the last time in isotonic solution of sodium chloride, the original volume being restored. The resulting suspension of organisms constitutes the "live washed meningococci" used in all three parts (A, B and C) of experiment 1. After removal of the required amount of material the remainder of the suspension was heated for fifteen minutes at 60 C., resulting in death of the bacteria. This constitutes the "dead washed meningococci." The inoculation of materials thus produced was always carried out within a few minutes after their preparation.

Each step of the procedures was controlled by culture of the inoculums just before use, insuring respectively the sterility of the material and the viability or the death of the organisms.

Supernatant β (table 1) was made up in one batch by a modification of Shwartzman's method. The meningococci were grown on dextrose-veal-agar in Kolle flasks for twenty-four hours, and the growth of each flask washed off with 3 cc. of isotonic solution of sodium chloride. The pooled washings were centrifuged until the supernatant was clear. It was then decanted, heated for fifteen minutes at 60 C. and stored in stoppered sterile bottles. Its potency was tested by producing the typical Shwartzman reaction in a number of rabbits. No attempt was made to titrate the strength of the material.

Each of the three parts of experiment 1, A, B and C, as illustrated in table 1, was carried out on 15 rabbits. The animals, not selected by sex or subspecies,

9. Shwartzman, G.: Proc. Soc. Exper. Biol. & Med. **26**:207, 1929.

averaged between 5 and 7.5 pounds (2 and 3 Kg.) and were divided into three groups of 5: E, F and G.

Approximately twenty-four hours before the first inoculation, the abdominal hair of the rabbits was clipped and then depilated with barium sulfide.

The intradermal inoculation, in all instances 0.15 cc. of material, was followed approximately twenty-four hours later with an intravenous injection of 1 cc. of material. The results of the experiment are illustrated in table 1.

Results.—Three animals, one each of groups BG, CE and CG, were excluded because of imperfect intravenous injections. Characteristic cutaneous lesions are depicted in figure 1.

Reading the results horizontally (table 1), i. e., AE, AF and AG, permits comparison of the preparatory potency of the various substances.

TABLE 1 *(Experiment).—Table Showing That the Local Shwartzman Reaction May Be Prepared and Provoked in Rabbits by Living, as Well as Dead, Meningococci or Their Supernatant Fluid*

	Rabbit Group E		Rabbit Group F		Rabbit Group G	
	Live Washed Meningococci	Reaction	Sterile Supernatant Fluid	Reaction	Dead Washed Meningococci	Reaction
Part A	i.d. Meningococci	+	i.d. Supernatant α	0	i.d. Meningococci	+
		+		0		+
		+		0		+
	i.v. Supernatant β	+	i.v. Supernatant β	0	i.v. Supernatant β	+
		+		0		⊕
Part B	i.d. Supernatant β	⊕	i.d. Supernatant β	0	i.d. Supernatant β	+
		0		+		+
	i.v. Meningococci	+	i.v. Supernatant α	+	i.v. Meningococci	+
		+		+		+
		I II		I		I II
Part C	i.d. Meningococci	+	i.d. Supernatant α	0	i.d. Meningococci	0
		0 +		0		0 0
		0 +		0		0 +
	i.v. Meningococci	+ +	i.v. Supernatant α	0	i.v. Meningococci	0 +
		+ +		0		+ +

i.d. = intradermal inoculation (preparatory).
i.v. = intravenous inoculation (provocatory).
\+ = Shwartzman reaction.
0 = no Shwartzman reaction.
⊕ = rabbit died too soon for reaction to manifest itself.
I = original experiment.
II = check experiment.

It is apparent that living and dead washed meningococci are capable of preparing the skin of rabbits, while the sterile supernatant is not. In a further experiment, not included in this paper, it was demonstrated that this inability is due not to the absence of the preparatory factor but to its low concentration in the fluid.

BE, BF and BG show that all three materials may provoke the phenomenon Since Shwartzman [6] has demonstrated that meningococcic filtrates are more potent as provocative than as preparatory agents, the results are in keeping with expectations.

E and G of experiment C were repeated and are labeled, respectively, CE II and CG II. The washed organisms, both living and dead, are demonstrably capable of preparing and eliciting the Shwartzman phe-

nomenon without the agency of filtrates. As in the case of AF, repetition of CF, with more concentrated preparations being utilized, resulted in successful production of the phenomenon.

Reactions produced with living organisms appeared, on the whole, somewhat larger and more intense than those elicited with dead meningococci. These results support those of Pabst and Branham,[10] who found that heating potent filtrates, even at moderately low temperatures, appeared to decrease their potency.

While gross examination of the various lesions shows no appreciable differences other than those of size, section through the centers reveals

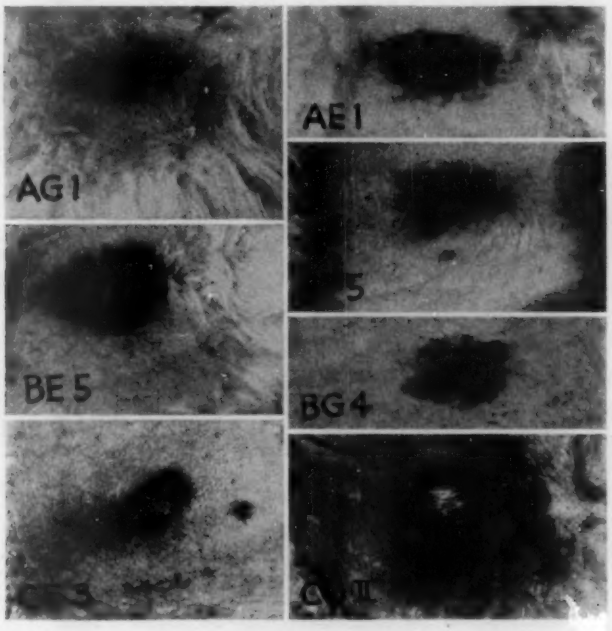

Fig. 1 (experiment 1).—Cutaneous Shwartzman reactions illustrating table 1. Each reaction is designated by reference to the part of the experiment (A, B or C) and to the rabbit group (E, F or G) and to the individual rabbit (1 to 5).

to the unaided eye that in many of the lesions of groups AE, AG, CE and CG an abscess has formed in the corium at its junction with the subcutaneous fat. Histologically (fig. 2) the cavity is crowded with polymorphonuclear leukocytes and some macrophages. The surrounding tissues are edematous and infiltrated by polymorphonuclear leukocytes. Adjacent blood vessels, both veins and arteries, are involved by the inflammation, presenting characteristic thrombophlebitis and thromoarteritis. Hemorrhage of varying degree is prominent. In almost

10. Pabst, A. M., and Branham, S. E.: Pub. Health Rep. **48**:639, 1933.

all instances the covering epidermis is still present but shows edema and an occasional infiltrating leukocyte.

The lesions produced in subgroups BE, BF and BG are similar in all respects to those described except for the absence of any but microabscesses. Acute cellulitis and thrombophlebitis and thromboarteritis with hemorrhage are seen in all positive reactors. The lesions are identical with those described by Shwartzman,[6] Apitz,[11] Moritz [12] and others and require no further description.

At necropsy 1 animal was selected from each group for more complete histologic study. Sections were removed from heart, lungs, liver,

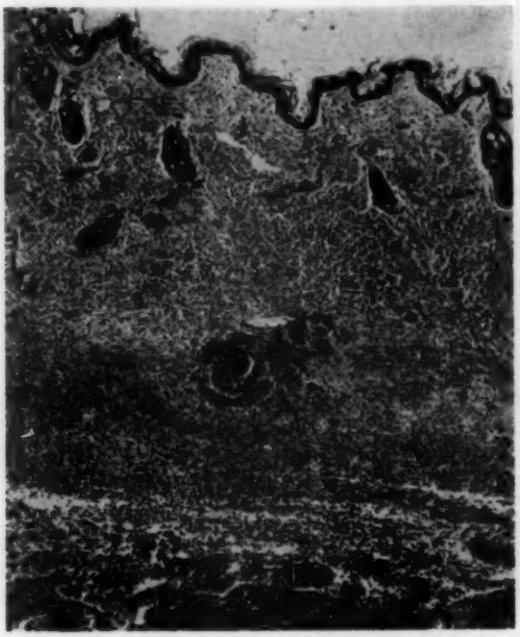

Fig. 2.—Cellulitis, thrombophlebitis and abscess formation characterizing the local cutaneous Shwartzman reaction (experiment 1); × 52.

spleen, kidneys and adrenal glands. The spleen showed the most consistent changes; the lymphoid follicles were enlarged and in many instances infiltrated by groups of polymorphonuclear leukocytes, among which were numerous pseudoeosinophilic leukocytes. Macrophages filled with cellular debris were likewise seen within the follicles (fig. 3*A*). The sinusoids, particularly those about the follicles, contained numerous polymorphonuclear leukocytes and occasional thrombi. They

11. Apitz, K.: Ztschr. f. d. ges. exper. Med. **89**:699, 1933.
12. Moritz, A. R.: J. Exper. Med. **66**:603, 1937.

were lined by hyperplastic reticuloendothelial cells. Since these changes manifested themselves eccentrically about the follicles, the result was a form of inflammatory crescent. The changes encountered in the other organs, with one exception, were similar to those described in great detail by Apitz,[13] Shwartzman, Klemperer and Gerber[14] and Gerber,[15] namely, occasional foci of acute myocarditis and necrosis (fig. 3 *B*) and focal necrosis of the liver (fig. 3 *C*). The lung of rabbit CE_4 presented the exception noted. Figure 3 *D* depicts a small pulmonary artery showing an unusual type of arteritis. A segment of the vessel

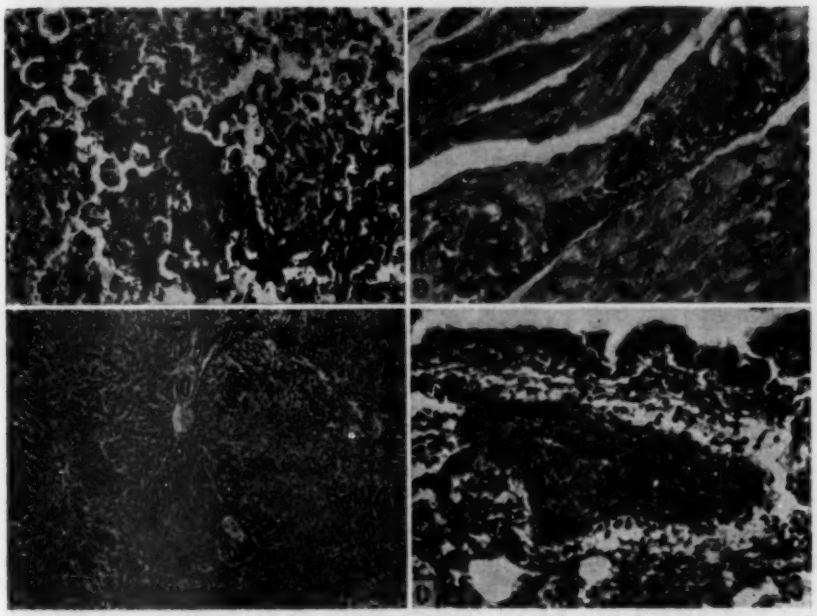

Fig. 3.—Visceral lesions accompanying production of the local cutaneous Shwartzman phenomenon (experiment 1); × 181. *A*, hyperplastic splenic follicle revealing debris-laden macrophages and infiltrating polymorphonuclear leukocytes. *B*, necrosis of myocardial fibers. *C*, focal hepatic necrosis. *D*, pulmonary arteritis.

reveals necrotic hyaline muscularis infiltrated by monocytes, among which are polymorphonuclear leukocytes. The intima is thickened by the presence of the same cell types. These obstruct the lumen. The adventitia and the periadventitia are likewise the site of a similar exudate.

13. Apitz, K.: Virchows Arch. f. path. Anat. **293**:1, 1934.

14. Shwartzman, G.; Klemperer, P., and Gerber, I. E.: J. A. M. A. **107**: 1946, 1936.

15. Gerber, I. E.: Arch. Path. **21**:776, 1936.

EXPERIMENT 2

Method.—While the method of Shwartzman is, beyond doubt, effective in estimating the potency of a given bacterial filtrate, it appeared to be of dubious value as a means of comparing the potency of one strain of meningococci with another. With different strains grown under uniform conditions there resulted at times gross discrepancies in estimates of numerical population. Since the potency of a given filtrate is directly related to the number of organisms in the culture, it was decided to utilize quantitative methods for assaying the potency of 18 meningococcic strains collected from various sources. Dead bacteria were used throughout (experiment 1). To obtain approximately equal numbers of organisms the following experiment was carried out: Meningococci of a single strain were planted on three dextrose-starch-agar slants and incubated for twenty-four hours. The growth was then washed off with 1 cc. of sterile saline solution. The organisms in the pooled washings were promptly killed by heating at 60 C. for fifteen minutes and twice washed as in experiment 1. After the last supernatant fluid had been decanted, 2 cc. of isotonic solution of sodium chloride was adde, and the organisms were resuspended and centrifuged

TABLE 2 *(Experiment 2).—Production of Purpurogenic Substance by Eighteen Meningococcic Strains*

Potency > P_1	Series Group	Potency = P_1	Series Group	Potency < P_1	Series Group
BG_1●	II	P_1●	II	D_1●	II
BG_2●	I	F_1●	I	W_1○	II
G_1●	IIa	D_4○	II	71○	I
D_3●	I	56○	II	54○	II
P_3●	I	G_2○	IIa	58○	I
44B	III	70○	I		
		G_3○	I		

● = organisms were recovered from cases of meningococcic purpura.
○ = organisms were recovered from cases of nonpurpuric meningitis.
44B = history unknown.

at high speed for two minutes in order to throw down aggregates of organisms and particles of culture medium. The supernatant suspension of meningococci was then pipetted into washed cuvettes, placed in a photronreflectometer and diluted with saline solution until a previously determined setting (maintained throughout the experiment) was reached. The resultant suspension was labeled 1. Four dilutions of this were prepared, each one-half the concentration of the preceding; they were labeled respectively 2, 3, 4 and 5. Thus, the concentrations of the materials for inoculation represented one-half, one-fourth, one-eighth and one-sixteenth meningococcic suspension 1.

Groups of 4 rabbits were used for the titration of each strain. The animals were clipped and depilated approximately twenty-four hours before use. Each received two series of five injections, the highest concentration most cephalad, 0.15 cc. being the standard dose. In each animal throughout the entire experiment the left hand series consisted of strain P_1; the right, of the strain being compared to P_1. Twenty-four hours after the preparation of the skin each animal received intravenously 1 cc. of potent supernatant fluid. The prepared sites were examined periodically, most of the lesions appearing within two hours. On one occasion an animal reacted within twenty minutes; frequently the lesions appeared within one hour.

Results.—The results of the titrations carried out on 18 strains of meningococci, 17 associated with known meningococcic purpura or non-purpuric meningitis, are recorded in table 2.

The production of the purpurogenic substance was measured only in terms of strain P_1. Whether, for example, D_1 is a greater producer than W_1 was not determined. The differences in some instances were small but always definite; when doubt as to classification arose, the strain was considered to be equipotent to P_1.

Strain 44B was supplied through the kindness of Dr. G. Shwartzman. The potency of its filtrates has been determined by Shwartzman's method through the course of years and found to vary between 600 and 4,000 reacting units per cubic centimeter. Whether it was originally associated with cutaneous purpura is unknown.[16]

As seen in table 2, of 8 strains of meningococci isolated from purpuric meningococcemia, 5 fall into the group of greatest producers along with 44B, 2 into the intermediate category, and only 1 into the group of relatively poor producers. Five of the 9 strains from non-purpuric meningitis are equal to P_1, and 4 are less potent.

All animals were killed within three days after the intravenous inoculation. The organs of those showing gross lesions were sectioned and examined histologically. In both series P_1D_1 and P_1F_1 an animal showed bilateral adrenal hemorrhages. The glands of P_1D_1 were swollen to at least twice normal size and were purple (fig. 4 *A*). The cortices of the organs were seen microscopically to be almost entirely necrotic and hemorrhagic. In a manner completely analogous to bilateral renal cortical necrosis a narrow margin of the zona glomerulosa subjacent to the capsule was intact around the entire periphery of the gland. There were zones of polymorphonuclear leukocytes and erythrocytes demarcating the lesions, many of which showed well advanced ischemic necrosis. The capillaries were only occasionally filled with homogeneous clots, conglutination thrombi. So small was their number that it was impossible to believe that they played a significant role in the production of the lesions. In other sites the damage of the adrenal parenchyma was less marked; here vacuolation of the cytoplasm and early pyknosis of the nuclei testified to the damage already wrought. In both glands the medulla was only moderately involved; some of the veins and arteries were thrombosed.

Study of the adrenal glands of animal P_1F_1 showed a similar but less advanced process. The necrosis again was of the ischemic type, outlined by a zone of hemorrhage. The polymorphonuclear leukocyte response was absent, and only the zona fascicularis was involved. The zona glomerulosa and the zona reticularis, as well as the medulla, were intact. The vessels of the medulla were all patent (fig. 4 *B*).

16. Shwartzman, G.: Personal communication to the authors.

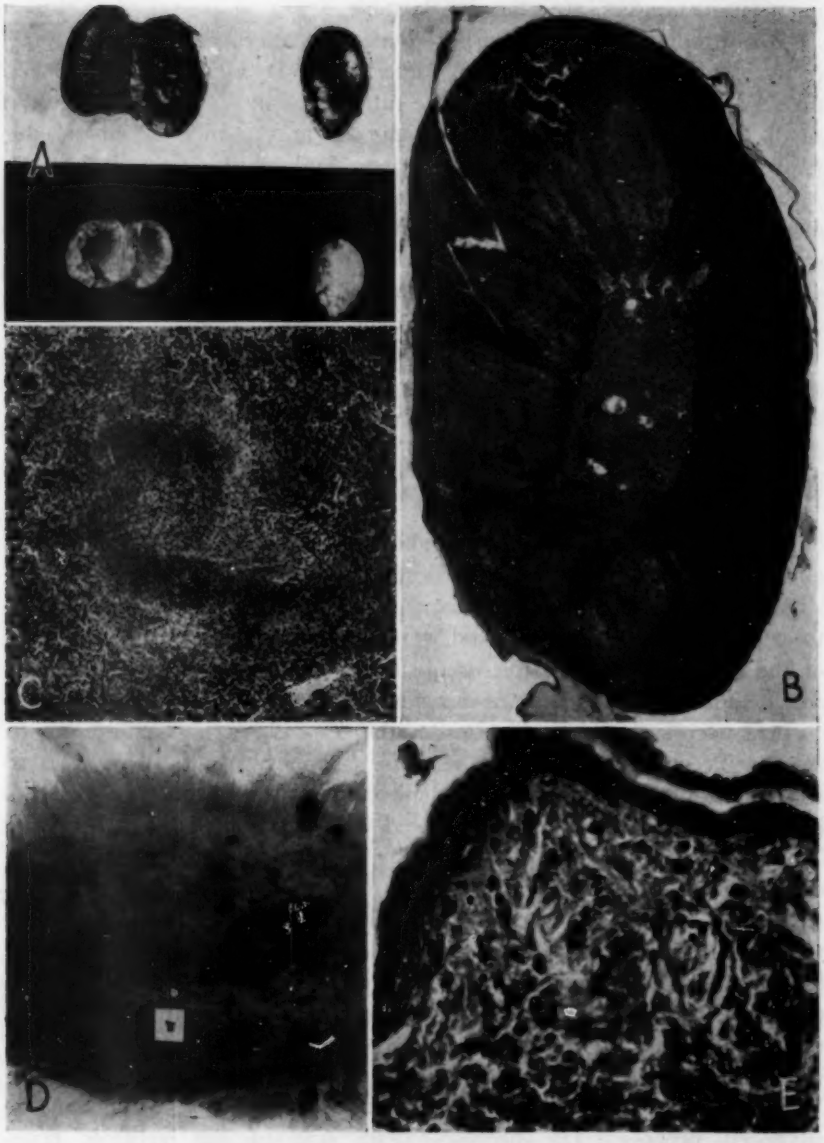

Fig. 4.—*A*, necrosis and hemorrhage of adrenal glands accompanying titration of meningococcus strain D_1 against P_1 (experiment 2); the lower pair of glands are normal. *B*, adrenal gland showing necrosis in areas of the cortex (paler areas); × 52. The medulla is intact. (Animal P_1F_1 in experiment 2.) *C*, focus of ischemic necrosis of the spleen (experiment 2); × 52. *D*, Shwartzman reactions at sites of intradermal inoculation of meningococci and cutaneous purpura provoked by meningococcemia (experiment 3). *E*, cutaneous hemorrhage provoked by meningococcemia (experiment 3); × 250.

Aside from small foci of necrosis in the myocardium of P_1D_1 the spleen and the liver revealed the only visible lesions. The splenic sinusoids in both instances were occluded by thrombi which in some areas were so numerous as to result in small foci of infarction (fig. 4 *C*). The central zones of the hepatic lobules were necrotic; this was especially noticeable in P_1F_1. The portal fields were not remarkable.

EXPERIMENT 3

Methods.—To test the response of rabbits to sustained meningococcemia a saline suspension of twice-washed living meningococci (strain P_1) was prepared as described in experiment 1 and administered intravenously every three hours in doses of 0.5 cc. to groups of 4 rabbits. For the fourth and eighth dose fresh

TABLE 3 *(Experiment 3).—Production of Meningococcemia and the Related Observations*

Meningococcus	Rabbit	Intravenous Injections	Local Shwartzman Reaction	Diffuse Cutaneous Purpura	Adrenal Necrosis	Bilateral Renal Cortical Necrosis
P_1	1a*	5	+	+	+	0
	2a	7	0	0	0	0
	3a	7	+	0	0	0
	4a	8	+	+	0	+
P_1	1b	4	0	0	0	0
	2b	6	0	0	0	0
	3b	6	0	0	0	+
	4b	6	+	+	0	0
D_3	1c	3	..	0	0	0
	2c	3	..	0	0	0
	3c	5	..	0	0	+
	4c	6	..	+	0	+
P_1	1d	5	..	0	0	0
	2d	7	..	0	0	+
	3d	7	..	+	0	+
	4d	8	..	0	0	+

* This rabbit presented the Waterhouse-Friderichsen syndrome.
\+ The lesion was present.
0 No lesion developed.

material was prepared, and between inoculations the organisms were refrigerated to reduce the danger of contamination.

To provide a field for observation the abdominal hair was clipped and in some groups depilated with barium sulfide approximately twenty-four hours before the experiments were undertaken.

The animals of the first group, labeled "a" in table 3, received two intradermal abdominal depots of the same suspension of P_1 immediately after the first intravenous injection. The animals of group b were treated in an identical manner except that 1 intradermal depot was made rather than 2. Groups c and d received living meningococci intravenously as already outlined without any intradermal depots. Strain D_3 was used instead of P_1 in group c because at the time it was more readily available.

Results.—An unexpected observation was the development of a bleeding tendency in all 16 animals. This tendency became apparent after

the second or third injection as perivenous hemorrhages about inoculation sites and prolonged bleeding time. Hematologic investigations were not undertaken but are to be carried out in the near future.

Animal 1a died some time within the three hours after the fifth inoculation, fifteen to eighteen hours after the experiment was begun. In this period not only did a typical Shwartzman reaction appear at the site of each intradermal depot (fig. 4 *D*) but purpura clearly manifested itself within the depilated abdominal zone. The cutaneous manifestation of meningococcemia was also seen over both flanks and lateral abdominal walls after the hair of the dead animal had been clipped. The petechiae varied from submicroscopic size to about 5 mm. in greatest dimension. The largest were produced in part by confluence of the smaller. They were all purple-red, were not raised above the niveau of the surrounding skin and were particularly numerous on the surfaces of the posterior and anterior flanks.

At necropsy both adrenal glands were at least one and a half times the usual size and flecked with hemorrhage. The remaining organs did not appear remarkable except for occasional small subserosal hemorrhages.

Histologic examination of the heart and the kidneys revealed no recognizable lesions. Some groups of pulmonary alveoli were filled with transudate. The liver was the site of early and definite centrolobular necrosis, and the splenic sinusoids contained occasional thrombi. The adrenal glands showed the same type of focal necrosis with surrounding zones of hemorrhage and well defined areas of early degeneration as were described in experiment 2. The skin presented the only new aspects. In the papillae and the dermis adjacent to the squamous epithelium were foci of hemorrhage (fig. 4 *E*). About some of these were collections of lymphocytes and only few monocytes. The deeper vessels were filled with blood; however, no evidence of thrombosis was visible in any of the sections studied. The zone of Shwartzman reaction showed the changes described in experiment 1.

In animal 3a, as indicated in table 5, only the site of intradermal inoculation reacted in the usual manner. In animal 4a, on the other hand, purpura developed, as well as a localized reaction, similar to but not as marked as that in 1a.

Only animal 4b of group b reacted; in this instance the response was similar to that of 4a, namely, generalized purpura and purpura at the site of intradermal inoculation.

Among the two groups receiving living organisms only intravenously, animals 4c (fig. 5 *A*) and 3d presented the characteristic generalized purpura.

An interesting observation was the appearance of typical bilateral cortical necrosis of the kidneys in 4a, 3b, 3c, 4c, 2d, 3d and 4d (fig. 5 *B*).

The kidneys were examined histologically. In all instances the characteristic necrosis of the glomeruli and the convoluted tubules was seen (fig. 5 *C*). Many of the glomerular capillaries were filled with conglutinated erythrocytes; in 3b fibrin strands were also present. The necrotic tissue was divided into small areas by zones of infiltrating polymorphonuclear leukocytes. These areas, however, were not necessarily separated by intervening segments of preserved kidney. Indeed, the

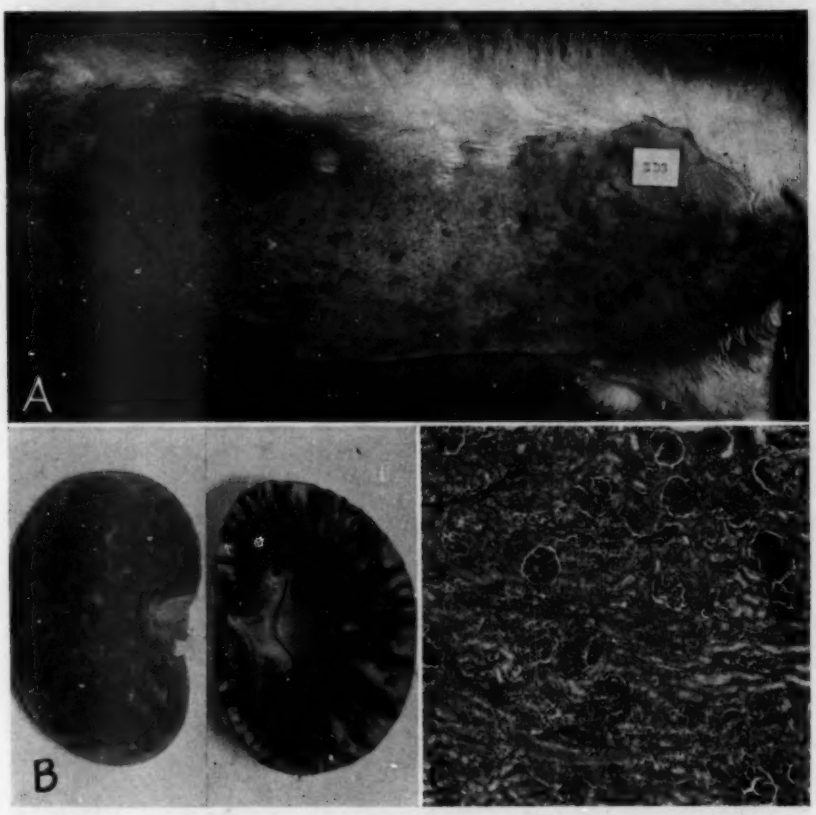

Fig. 5.—*A*, purpuric meningococcemia (experiment 3). *B*, bilateral cortical renal necrosis produced by meningococcemia (experiment 3). In some areas the outer part of the medulla is involved. *C*, necrosis of the renal cortex (experiment 3); × 52.

necrosis sometimes extended almost uninterruptedly over the entire cortex, the older areas of necrosis joined by fresher zones of devastated renal tissue. The arcuate arteries and their larger branches were singularly free from thrombi (fig. 6 *A*). In 3b a few fibrin-containing thrombi were demonstrable (fig. 6 *B*); for the remainder the only evidence of obstruction was conglutinated erythrocytes. In all kidneys

except those of 3d the walls of the interlobular arteries and the afferent arterioles were necrotic. An interesting observation in 4a was necrosis of Henle's loops within the outer zone of the medulla (fig. 5 *B*). The kidneys of animal 3d represented the earliest stage in the evolution of the lesion. The glomeruli and the convoluted tubules were already

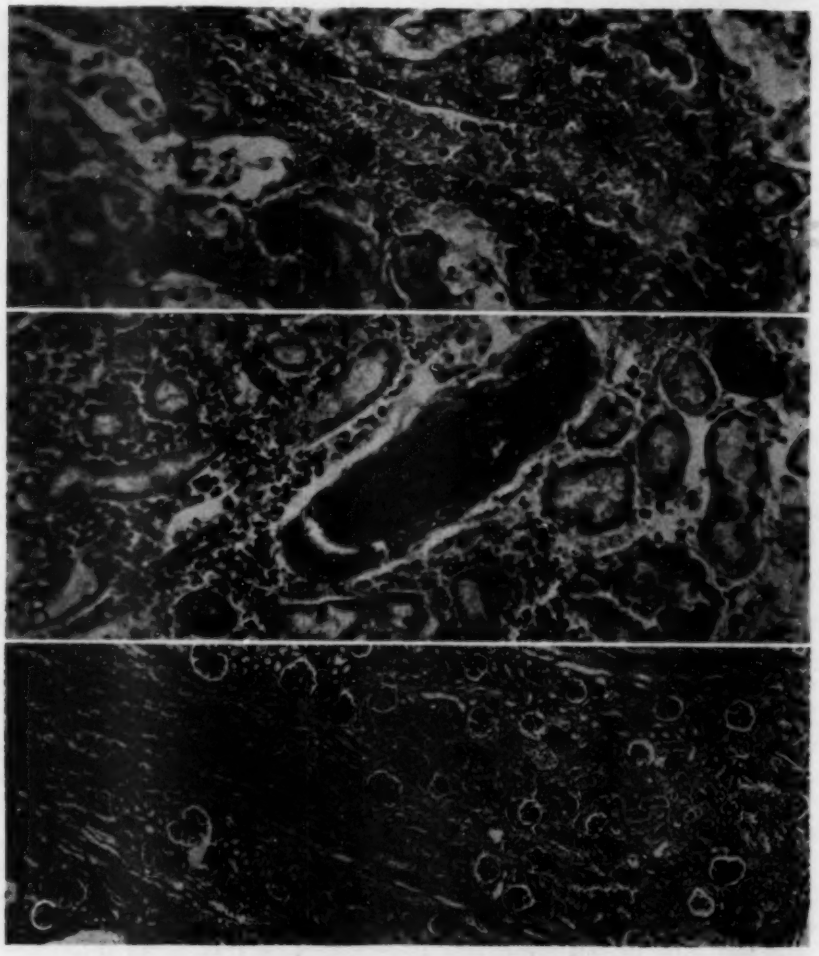

Fig. 6.—*A* and *B*, higher power views of renal cortical necrosis (experiment 3). *A* shows a characteristically patent, although necrotic, interlobular artery; × 236. *B* shows a necrotic interlobular artery filled with thrombus; × 236. *C*, kidney of a killed rabbit showing a focus of nonfatal typical cortical necrosis; × 49.

necrotic, although the structural details were still clearly visible. The leukocytic demarcation line was only barely visible here and there. Conglutination thrombi were seen within capillary lumens in both the

glomerular loops and the cortical interstices. The interlobular arteries and branches were unobstructed and their walls as yet apparently intact.

All histologic preparations of the 4 animals were stained for bacteria by MacCallum's modification of Goodpasture's method [17] and the Brown-Brenn technic.[18] In no instance was it possible to demonstrate the definitive presence of either intracellular or intercellular gram-negative diplococci. It is true that coccoid particles were seen, but because of the large number of pseudoeosinophilic leukocytes and the presence of cellular debris it was impossible to ascertain their identity. Since meningococci have been clearly demonstrated in blood smears from cutaneous purpura of meningococcemia stained by Gram's method, the absence of organisms in the experimental lesions was investigated. For this purpose 2 rabbits received a number of intradermal inoculations, each consisting of 0.15 cc. of a heavy suspension of gram-negative diplococci (G_2). The lesions following the inoculations were removed under ether anesthesia at the following intervals: one hour and fifteen minutes, six hours and thirty minutes, ten hours, fifteen hours and twenty-four hours and thirty minutes. Each specimen was fixed in Zenker's fluid, and sections were stained with simple methylene blue and by MacCollum's modification of Goodpasture's method, the Brown-Brenn method and the Glynn modification of Gram's method.[17] In the methylene blue preparation of the first specimen masses of recognizable diplococci were seen. MacCollum's and Glynn's stains showed them as faintly gram-negative particles, recognizable as meningococci only because of the knowledge of their location gained from studying the first section. In the Brown-Brenn preparation the organisms could not be seen. The second specimen removed revealed in the MacCollum, Glynn and Brown-Brenn stained sections no identifiable gram-negative diplococci. Meningococci were, however, presumed to be recognized in the methylene blue preparation.

The same results were found in the remaining three specimens. With the progressive accumulation of leukocytes and the appearance of cellular detritus, organisms were even more difficult to identify.

This signal failure to differentiate meningococci in rabbit tissue by means of the usual tinctorial methods after large numbers of demonstrable gram-negative diplococci were inoculated intradermally is a pregnant comment on the difficulty of staining, as well as of the recognition of, Neisseria in fixed tissues. Push's report [19] exemplifies this difficulty in contrast to the easy success which others have reported.

17. Mallory, F. B.: Pathological Technique, Philadelphia, W. B. Saunders Company, 1938.

18. Schaub, I. G., and Foley, M. K.: Methods for Diagnostic Bacteriology, St. Louis, C. V. Mosby Company, 1943.

19. Thomas, H. B., and Leiphart, C. D.: J. A. M. A. **125**:884, 1945.

One animal from each of groups a and c showed the same type of pulmonary arteritis described in rabbit CE_4 of experiment 2.

MENINGOCOCCIC PURPURA

In the past relatively few attempts have been made to test the ability of living or dead bacteria to produce the Shwartzman phenomenon. The work of several authors [20] employing unwashed cultures may not be considered, since the inoculums may have contained besides the organisms relatively large amounts of soluble potent substances.

Witebsky and Salm,[21] working with washed suspensions of Haemophilus influenzae, Moritz [12] with Salmonella aertrycke and Witebsky and Neter [22] with Pneumococcus were able to demonstrate the ability of their respective bacteria to prepare the skin and/or elicit the phenomenon. Pneumococcus possessed only the power to prepare skin.

Two groups of investigators employed meningococci in their experiments. Riley and Wilson [23] in their study of the toxicity of various meningococcic preparations successfully used heat-killed washed meningococci to prepare the site and evoke the Shwartzman phenomenon. Schneirerson [24] prepared the skin of rabbits with living washed, as well as dead dried, meningococci. He found that while the living cocci could provoke the reaction the heat-killed meningococci could not.

It appears, then, that the experiments reported in table 1 confirm in full the work of Riley and Wilson and of Schneirerson, except for the latter's inability to provoke the phenomenon with dead dried organisms. Since Schneirerson heated the meningococci for one hour at 56 C., in contrast to our fifteen minutes at 60 C., his failure may have been due to heat inactivation of the potent principle, despite its relative heat stability.

The design of experiment 1 is such as to make possible a critical statement concerning Shwartzman's assertion [6] that the filtrate of a given culture contains much more of the potent materials than may be found in the disintegrate of its organisms. In our hands the washed meningococci, both living and dead, are far more potent than the supernatant, which corresponds to Shwartzman's filtrate.

Assuming that the 18 strains are a representative sample of all pathogenic meningococci, simple inspection of table 2 makes it apparent

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20. Sanarelli, G.: Ann. Inst. Pasteur **38**:11, 1924. Gratia, A., and Linz, R.: Compt. rend. Soc. de biol. **107**:1579, 1931. Koplik, L. H.: Am. J. Path. **11**:842, 1935. Alechinsky, A., and Renaux, E.: Compt. rend Soc. de biol. **128**:790, 1938. Alechinsky, A.: ibid. **129**:513, 1938. Shwartzman. [6]

21. Witebsky, E., and Salm, H.: Proc. Soc. Exper. Biol. & Med. **34**:351, 1936.

22. Witebsky, E., and Neter, E.: Proc. Soc. Exper. Biol. & Med. **38**:187, 1938.

23. Riley, C. V., and Wilson, M. A.: J. Immunol. **23**:269, 1932.

24. Schneirerson, S. S.: J. Infect. Dis. **65**:97, 1939.

that all strains of Neisseria meningitidis causing human disease are potentially capable of eliciting cutaneous purpura since in all three groups such organisms are present.

It is believed that the purpurogenic substance is identical in all strains, varying only quantitatively among the organisms of the three categories of table 2. Since in the first column 5 strains are associated with purpura and none of the 9 nonpurpuric strains falls into this group, it appeared desirable to recast the results in the form of a contingency table by combining the central and right columns of table 2. Of 5 strains with a potency greater than P_1, all were from instances of purpuric meningococcemia. The potency of none of the 9 strains isolated from cases of nonpurpuric meningitis was greater than that of P_1. The probability of such a distribution being a matter of chance is 8 per cent (restricted point binomial method). While this result does not establish a statistically significant distribution, the trend in this small group is such as to make inescapable the conclusion that the presence or the absence of cutaneous purpura in meningococcic disease is in part dictated by the strain of Neisseria causing the infection.

That this factor, however, is not solely responsible for purpuric meningococcemia is evident. P_1, F_1 and D_1, table 2, while associated with cutaneous purpura, are nevertheless equipotent only with the 9 nonpurpuric strains. It follows, therefore, that in some instances a factor or factors other than the purpurogenic substance play a decisive role.

Since meningococci may be divided into four serologic groups, the possible relationship of these innate differences to the presence or the absence of purpura in the human sources of the organisms was investigated. Table 2 clearly shows the absence of any such relationship. However, an observation made in all three experiments, embracing a total of 125 rabbits, appears pertinent to the discussion, namely, the factor of individual reactivity. As may be seen by examination of tables 1 and 3, while some animals react, others do not to the almost simultaneous inoculation of living or dead meningococci prepared at the same time and obtained from the same source. Because of this, it was impossible to predict in detail the outcome of any particular experiment.

The assay of strain F_1 against P_1, stylized in figure 7, illustrates this factor. Rabbit 1 did not react in any of the prepared sites. In contrast, animal 2 responded in all P_1 and four of the F_1 sites. Animals 3 and 4 show reverse images of their reactions, animal 3 reacting in the first two and three sites of F_1 and P_1, respectively, and animal 4 in the first three and two sites of F_1 and P_1.

Drawing, therefore, an analogy between the rabbits and man, it may be said that meningococcic purpura is most apt to appear through

the fortuitous infection of a person with marked susceptibility to the Shwartzman toxin by a very purpurogenic meningococcus.

The individual variability and perhaps the meningococcus strain likewise appear to determine the time required for reaction following the provocative inoculation. In the assay of P_2 1 animal responded in all ten sites twenty minutes after the intravenous injection. Many animals showed reactions after one hour; others, only after two or three hours.

Experiment 3 is the logical sequel to experiments 1 and 2 and represents an attempt to produce generalized meningococcic purpura in a manner similar to that in human subjects. Not recorded in table 3 is the fact that the Shwartzman reactions at the sites of intradermal inoculation in animals 1a (fig. 4 *D*), 4a and 4b coincided with the

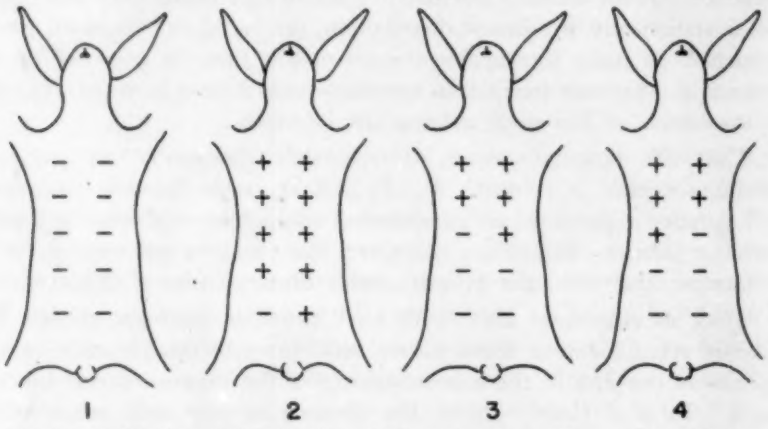

Fig. 7.—Stylization of the titration of the preparatory potency of meningococcus strain F_1 (left) against that of strain P_1 (right) (experiment 2).

appearance of the general cutaneous purpura. The converse of the foregoing statement is likewise significant, namely, that in no instance did general purpura appear without a corresponding reaction in the intradermal depot. These observations strongly suggest that genetically both lesions are closely related, if not identical.

Animal 3a, which had a weakly positive Shwartzman reaction about the intradermally deposited meningococci, did not suffer general purpura despite its seven intravenous inoculations of living cocci. Since the extent of a local Shwartzman reaction in a given animal is directly related to the amount of potent material inoculated, it follows that of the two (local reaction about the depot and general purpura) the former is more apt to occur, since more meningococci have been inoculated than could possibly reach an equal area of skin through the blood stream.

The c and d series of rabbits received only intravenous inoculations of living organisms, and characteristic cutaneous purpura (fig. 5 *A*)

developed in 2 rabbits, 4c and 3d, confirming the results of groups a and b.

If it is assumed, therefore, that each cutaneous lesion represents a Shwartzman reaction in miniature, the following interpretation of meningococcic purpura appears warranted. The human disease is most frequently preceded by an infection of the upper respiratory tract, from which locus, it is believed, meningococci enter the blood stream and multiply. Neisseria and their products are deposited in the skin and prepare it. The time necessary to achieve this varies with the patient's reactivity and the strain of the infecting meningococcus. When the incubation time is spent, the patient, displaying meanwhile the symptoms of continuing bacteremia, suddenly presents the first purpuric lesions. These are produced by the provocatory effect of the bacteremia. The number and the size of the lesions depend again on the two factors just mentioned: the patient's reactivity and the potency of the infecting strain of meningococcus. Thus, in some subjects, human and rabbit, no lesions develop; in others a few petechias, and in still others massive confluent purpuric areas. The continuing appearance of the lesions is due to the further provocation of foci established later in the course of the bacteremia.

It is understood that the foregoing statement represents a working hypothesis which remains to be confirmed by a study of the effects of meningococcemia on the constituents of blood and ultimately the isolation of the factor or factors responsible for the reaction.

THE WATERHOUSE-FRIDERICHSEN SYNDROME

The one microscopic and three macroscopic instances of adrenal necrosis and hemorrhage of experiments 2 and 3 raise the problem of the Waterhouse-Friderichsen syndrome.

It is well known that necrosis of the adrenal glands associated with varying degrees of hemorrhage may occur not only in a large number of infectious diseases (meningococcemia, diphtheria,[25] influenza[26] and others) but also in noninfectious states (toxemia of pregnancy,[27] severe burns,[28] as well as in the newborn[29]) and a host of more or less poorly understood conditions.[30] Experimental procedures of various types have likewise resulted in the production of adrenal necrosis and hemor-

25. MacLean, A.: J. Hyg. **37**:345, 1937.

26. Lucké, B.; Wight, T., and Kime, E.: Arch. Int. Med. **24**:154, 1919.

27. Dodds, G. H.: Brit. M. J. **1**:769, 1945

28. Harkins, H. N.: The Treatment of Burns, Springfield, Ill., Charles C Thomas, Publisher, 1942, chap. 6.

29. Goldzieher, M. A., and Murray, G. B.: Endocrinology **165**:181, 1932.

30. Mitchell, W., and Angrist, A.: Am. J. M. Sc. **205**:549, 1943.

rhage; administration of drugs,[31] bacteria and their products,[32] dietary deficiencies [33] and various forms of acute shock [34] are but a few of the methods utilized.

In all of the more authoritative reports the adrenal necrosis and hemorrhage have been in the zona fasciculata and to a lesser extent in the zonae glomerulosa and reticularis. The medulla is usually preserved. The descriptions of the glands, regardless of etiologic agent, correspond to that recorded in the protocols of this report. Whether a common factor underlies the common end result is a moot question. Hoerr [35] suggested, on the basis of Neuman's work,[36] that since the adrenal glands possess the greatest blood supply of all organs they must likewise receive a corresponding share of any circulating noxious material and are therefore more liable to injury. On the other hand, one must consider that an abundant circulation may be necessary to the survival of the adrenal parenchyma. If this is the case, moderate impairment of the general circulation may reflect itself in decreased adrenal function and severe impairment, perhaps in necrosis. Thus may be explained the adrenal necrosis of acute experimental hemorrhagic and dehydration shock. It appears therefore that pathologic states combining both possibilities—circulating toxic substances and circulatory collapse—should be more frequently associated with severe adrenal cortical damage. Human meningococcic disease may represent such a condition.

The incidence of purpuric meningococcemia varies from epidemic to epidemic, sometimes rising as high as 50 per cent.[37] In only a small number of the purpuric patients does adrenal necrosis develop. In analogy, only 2 of the 5 animals of table 3 with cutaneous purpura showed adrenal changes, 1a marked changes, 4a one microscopic focus. These results support the view that purpuric meningococcemia is not synonymous with the Waterhouse-Friderichsen syndrome, of which adrenal necrosis and hemorrhage are an integral part.

If meningococcemia is a disease which disposes toward adrenal damage, why do so few subjects show widespread adrenal cortical

31. (*a*) Humphreys, E. M., and Donaldson L.: Am. J. Path. **17**:767, 1941. (*b*) Reyna, F. G.: Beitr. z. path. Anat. u. z. allg. Path. **97**:268, 1936. (*c*) Flexner, S.: J. Exper. Med. **2**:197, 1897.

32. Olitsky, L.; Avinery, S., and Koch, P. K.: J. Immunol. **45**:237, 1942. Oppenheim, R., and Loeper, M.: Arch. de méd. expér. et d'anat. path. **13**:332, 1901. Gerber.[15]

33. Supplee, G. C.; Bender, R. C., and Kahlenberg, O. J.: Endocrinology **30**:355, 1942.

34. Davis, H. A.: Arch. Surg. **47**:939, 1941. Muirhead, E. E.; Ashworth, C. T.; Kregel, L. A., and Hill, J. M.: ibid. **45**:863, 1942.

35. Hoerr, N.: Am. J. Anat. **48**:139, 1931.

36. Neuman, K. O.: J. Physiol. **45**:188, 1912-1913.

37. Herrick, W. W.: J. A. M. A. **71**:612, 1918.

necrosis? This question suggests a third possible cause of the adrenal lesions.

Gerber [15] observed that a single intravenous inoculation of potent bacterial filtrate may produce unilateral adrenal lesions such as were encountered in the present series. Since it has been confirmed by thousands of experiments that the Shwartzman phenomenon may be produced only by first preparing tissue and then provoking the response via an intravenous inoculation, the adrenal necrosis may not be considered part of the Shwartzman phenomenon. The low incidence (2.4 per cent in 125 animals) of this series supports this point of view. The work of Gratia and Linz,[38] soon confirmed by Shwartzman and Michailovsky,[39] demonstrated the ability of one intravenous injection of potent filtrate to produce hemorrhagic necrosis of certain tumors. No satisfactory explanation has been forthcoming in elucidation of this remarkable phenomenon. The inference cannot be avoided that in some mammals, human and rabbit, the relationship of adrenal vasculature to adrenal parenchyma and the sensitivity of the structures to the Shwartzman toxin may be of the same order as that existing between the toxin and these tumors.

Since the adrenal lesion is an intrinsic part of the Waterhouse-Friderichsen syndrome but apparently not a result of the mechanism producing the cutaneous purpura (Shwartzman phenomenon), it appears fallacious to write of curing the syndrome, as so many have, when what is meant is the successful treatment of petechial meningococcemia. Until clinical means are devised for differentiating impairment of adrenal function from adrenal necrosis, definitive diagnosis of the Waterhouse-Friderichsen syndrome must remain within the province of the pathologist.

In support of their belief that the destruction of the adrenal cortex in meningococcemia is just another hemorrhage in a hemorrhagic disease and without significant effect on its further course, Thomas and Leiphart [19] pointed out that septicemic shock and death may occur without histologic evidence of widespread adrenal damage. Furthermore, removal of dogs' and rats' adrenal glands does not immediately result in death of the animals but is followed by a gradual decline over a period of days. While both statements are true, it does not appear reasonable to compare the result of adrenalectomy in a healthy animal with that of spontaneous destruction of the adrenal cortices in a desperately sick person. It would appear better judgment to assume that such a lesion would hasten, if not precipitate, the fatal termination of the illness.

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38. Gratia, A., and Linz, R.: Compt. rend. Soc. de biol. **108**:427, 1931.

39. Shwartzman, G., and Michailovsky, N.: Proc. Soc. Exper. Biol. & Med. **29**:737, 1932.

During the increase of meningococcic disease associated with World War II several authors [40] have stated more or less categorically that the sole cause of the Waterhouse-Friderichsen syndrome is meningococcemia. That such statements are intemperate is evident to students of the syndrome. In most recorded cases the bacteriologic studies made have been inadequate or unsuccessful, and in a small number of instances organisms other than meningococci have been demonstrated. One of us (Dr. Black-Schaffer) has made postmortem examinations of a number of persons with this syndrome. In 2 the syndrome undoubtedly was caused by, respectively, beta hemolytic streptococci and pneumococci.[41] Since the Shwartzman substance is not confined to the meningococcus but is found, albeit in lesser concentration, in other organisms, the occasional production of the syndrome by bacteria other than meningococci is not surprising.

BILATERAL RENAL CORTICAL NECROSIS

The bilateral cortial nerosis of the kidneys appearing in animals of experiment 3 was not entirely unexpected. Apitz [13] produced the lesion with two intravenous inoculations of potent bacterial filtrate, given twenty-four hours apart. This has since come to be recognized as characteristic of the generalized Shwartzman reaction. Since the living and dead washed meningococci have thus far been shown to possess properties similar to those of the corresponding bacterial filtrates and supernatant fluids, there was every reason to believe that an experiment reproducing the essentials of Apitz' work would achieve the same result: bilateral renal cortical necrosis.

In numerous experiments Apitz,[13] Gerber [15] and others have proved (with one exception to be discussed later) that the renal changes cannot be elicited, as can the local cutaneous reaction after suitable preparation, by a single intravenous inoculation of the Shwartzman substance.

Since washed living meningococci are able to produce this remarkable lesion when inoculated every three hours for a period of fifteen to twenty-four hours, the conclusion drawn from experiment 1—that meningococci possess the same properties as their filtrates—is buttressed. The concept of the mechanism of experimental meningococcic purpura and, by analogy, the human disease, first suggested by Shwartzman [6] and developed in this paper, is likewise reenforced. It clearly demonstrates the ability of the organisms of a continuous meningococcemia to prepare tissues and provoke the typical response, cutaneous purpura and/or bilateral renal cortical necrosis.

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40. Martland, H. S.: Arch. Path. **37**:147, 1944. Herbut and Nanges.[16] Thomas and Leiphart.[19]

41. Black-Schaffer, B.: Unpublished data.

Duff and Murray [42] in their otherwise excellent review of the subject of bilateral renal cortical necrosis unfortunately overlooked the generalized Shwartzman reaction. They reached the conclusion that the pathogenesis of the renal changes is intense vasospasm or vasoparalysis followed by necrosis of the interlobular arteries and their afferent arterioles, with accompanying stasis of circulation, possibly thrombus formation in some cases, and consequent ischemic necrosis of the renal cortex.

The lesions in the animals of experiment 3 were identical with those of symmetric renal cortical necrosis in man and animals, regardless of cause.

The pathologic anatomy of the kidneys studied in this series lends support to the hypothesis likewise supported by the work of Byrom,[43] deNavasque [44] and Penner and Bernheim.[45] The relative infrequency of other than conglutination thrombi and the necrosis of the arterioles and the interlobular arteries observed in the cases with older lesions fit well into the concept of primary vascular damage leading to circulatory stasis with subsequent conglutination of stagnant erythrocytes. In a preliminary report of recent date, Trueta and his colleagues [46] imparted the information that they had experimentally demonstrated a mechanism whereby the renal circulation is shunted directly from the larger arteries through the vasa rectae to the medullary veins, producing cortical ischemia. The mechanism is under neural control and may be elicited by a number of stimuli. They suggested that this shunt may play a considerable role in the production of the oliguria or anuria of trauma, puerperal accidents and other incidents. Such evidence, although not yet confirmed, pertinently supports the school of thought which insists on vasospasm as the cause of renal cortical necrosis.

A factor in our animals which at the present time cannot be adequately evaluated is the hemorrhagic diathesis previously mentioned but not discussed. The experimental production of necrosis of the renal cortices in 7 of 16 animals (experiment 3), all showing a bleeding tendency, favors the point of view that thrombosis of the renal vasculature may not be an important primary mechanism in the genesis of the condition.

The experimental and known toxicologic methods of producing bilateral cortical necrosis of the kidneys are varied. Intravenously

42. Duff, G. L., and Murray, E. G. D.: Am. J. M. Sc. **201**:428, 1941.

43. Byrom, F. B.: J. Path. & Bact. **45**:1, 1937.

44. deNavasque, S.: J. Path. & Bact. **46**:47, 1938.

45. Penner, A., and Bernheim, A. L.: Arch. Path. **30**:465, 1940.

46. Trueta, J.; Barclay, A. E.; Franklin, K. J.; Daniel, P., and Prichard, M. M. L.: Lancet **2**:237, 1946.

injected staphylococcic filtrates [47] have been employed to effect the changes in rabbits and cats. Repeated intravenous injections of hog cholera virus vaccine [48] likewise resulted in the appearance of the typical lesion. Simple chemical agents (intravenous lithium carmine in rabbits [31b] and dioxane,[49] diethylene glycol [50] and almond extract [51] poisoning in man) have also produced the renal lesion. The generalized Shwartzman reaction is part and parcel of this heterogeneous group of etiologic agents, whose mode of action is essentially unknown. In another category are the results of two experiments employing known vasospastic drugs: pitressin in rats [43] and epinephrine hydrochloride in dogs.[45] In these instances the characteristic lesion is ascribed to vasoconstriction with necrosis of the afferent arterioles and ischemia of the involved nephrons. Byrom [43] found, when large doses of pitressin were used, that the vascular necrosis extended to and involved the larger vessels of the kidneys. Penner and Bernheim [45] examined the literature of renal cortical necrosis and reviewed those striking cases in which a direct relationship appears to exist between traumatic or surgical shock and the onset of fatal urinary suppression in males and nonpregnant females. They concluded that in some human subjects the vasospasm of shock is sufficient to result in bilateral renal cortical necrosis.

The occurrence of the lesion in men and nonpregnant women is being reported more frequently.[42] It is now known that it may have a variety of initiating causes. Acute infections compose the largest group in this category. The results of our experiments do not permit conclusions on the relationship between acute infection and bilateral renal cortical necrosis. However, Gerber's cautious statement [18] may be reaffirmed: ". . . the changes observed in spontaneous and experimental infections in animals and in association with infection in man suggests that a similar pathogenesis may be operative." It is now demonstrated that not only extreme artefacts, such as those produced in the experiments of Apitz,[13] deNavasque,[44] Rigdon and co-workers [47a] and others, but a condition more closely approximating a natural infection, meningococcemia, is capable of eliciting the same results. In fact, the meningococcemia appears to be at least as effective in producing the changes as the inoculation of bacterial filtrates.

No report of bilateral renal cortical necrosis complicating meningococcic disease has appeared in the literature. This seems at variance

47. (*a*) Rigdon, R. H.; Joyner, A. L., and Ricketts, E. T.: Am. J. Path. **10**:425, 1934. (*b*) Von Glahn, W. C., and Wild, J. T.: J. Exper. Med. **61**:1, 1935. deNavasque.[44]

48. Röhrer, H.: Virchows Arch. f. path. Anat. **284**:203, 1932.

49. Barber, H.: Guy's Hosp. Rep. **84**:267, 1934.

50. Geiling, E. M. K., and Cannon, P. R.: J. A. M. A. **111**:919, 1938.

51. Garvin, C. F., and van Wezel, N.: Arch. Int. Med. **62**:423, 1938.

with the expectations of the preceding exposition and discussion. However, the extreme rarity of the condition in contrast to the everyday occurrence of all kinds of infections, the ability not only of meningococcic but of staphylococcic toxins and hog cholera virus to produce the lesion, and the complete absence of correlation of any particular infection with renal cortical necrosis make it abundantly evident that infection is of secondary importance in its genesis. These facts have led Duff and Murray [42] to assume that in certain apparently normal persons the interlobular arteries and glomerular arterioles may be hyperirritable.

The one exception mentioned in the foregoing discussion to the rule that renal cortical necrosis may not be produced with a single intravenous injection of Shwartzman filtrate is pregnancy.[13] Therefore, the fact that the most common etiologic moment in man is likewise pregnancy is of more than passing interest.

Three instances of postpartum necrosis of the renal cortices studied by one of us [52] revealed an important anamnestic factor: prepartum or intrapartum hemorrhagic shock associated with ablatio placentae. Perusal of the literature brings this dramatic prologue to the onset of uremia into the foreground. In 29 [53] of 50 reported cases of bilateral necrosis of the renal cortices complicating delivery this condition was preceded or accompanied by severe hemorrhage. The histories of 4 additional cases [54] were recorded in such a manner as to justify the interpretation that marked prepartum or intrapartum hemorrhage was present. In 1 other instance,[55] while hemorrhage is not mentioned, profound shock

52. Black-Schaffer, B.: Unpublished data.

53. (*a*) Bradford, J. R., and Lawrence, T. W. P.: J. Path. & Bact. **5**:189, 1898. (*b*) Cruickshank, J. N.: J. Obst. & Gynaec. Brit. Emp. **30**:336, 1923. (*c*) Dalrymple, S. C.: New England J. Med. **203**:160, 1930. (*d*) Davis, C. H.: J. A. M. A. **114**:2370, 1940. (*e*) Geipel, P.: Zentralbl. f. Gynäk. **38**:517, 1914. (*f*) Glynn, E. E., and Briggs, H.: J. Path. & Bact. **19**:321, 1914-1915. (*g*) Griffith, W. S. A., and Herringham, W. P.: ibid. **11**:237, 1906-1907. (*h*) Jardine, R., and Kennedy, A. M.: Lancet **1**:1291, 1913 (case 1). (*i*) Lloyd, H. C.: ibid. **1**:156, 1906. (*j*) Manley, J. R., and Kliman, F. E.: Am. J. Obst. & Gynec. **14**:802, 1927. (*k*) Reyna, F. G.: Beitr. z. path. Anat. u. z. allg. Path. **97**:268, 1936. (*l*) Scriber, W. deM., and Oertel, H.: J. Path. & Bact. **33**:1071, 1930. (*m*) Sheldon, W. H., and Hertig, A. T.: Arch. Path. **34**:866, 1942 (2 cases). (*n*) Stening, M. J. L.: J. Obst. & Gynaec. Brit. Emp. **46**:250, 1939. (*o*) Warner, C. G., and Hibbits, J. T.: Am. J. Obst & Gynec. **23**:875, 1935. (*p*) Westman, A.: Acta obst. et gynec. **7**:235, 1928 (case 1). (*q*) Tomlinson, W. J.: Am. J. Obst. & Gynec. **49**:236, 1945. (*r*) Davidson, J., and Turner, R. L.: Tr. Edinburgh Obst. Soc. **89**:101, 1929-1931 (cases 1 and 3). (*s*) Dunn, J. S., and Montgomery, G. S.: J. Path. & Bact. **52**:1, 1941 (6 cases). (*t*) Kellar, R. J., and Arnott, W. M.: Tr. Edinburgh Obst. Soc. **92-94**:101, 1932-1935 (cases 2 and 3).

54. Evans, N., and Gilbert, E. W.: Am. J. Path. **12**:553, 1936. Rolleston, H. D.: Lancet **2**:1173, 1913. Schüppel, A.: Arch. f. Gynäk. **103**:243, 1914. Torrens, J. A.: Lancet **1**:99, 1911.

55. Jardine, R., and Teacher, J. H.: J. Path. & Bact. **15**:137, 1910-1911 (case 2).

is said to have been evident. In 16 cases [56] hemorrhage or shock is said to have been absent or is not mentioned. Thus, of 53 women, 37 (69.8 per cent) were definitely the victims of severe shock, hemorrhagic or other, promptly followed by oliguria and uremia. It is impossible to estimate the real incidence, since most early authors regarded hemorrhagic shock as an unimportant, even though serious, complication. It is evident, therefore, that this figure represents a minimum incidence.

Many of the hemorrhages were of the ablatio (abruptio) placentae type. All textbooks of obstetrics are unanimous in asserting that the most important and constant symptom of this feared accident is profound shock, frequently entirely out of proportion to the blood lost. It is particularly in this type of obstetric accident that urinary suppression, with or without recovery, is most frequently encountered.

In modern times oxytocic substances, usually a pituitary extract or an ergot preparation, are used, either singly or together, in routine postpartum therapy. Two factors which are known vasospastic agents were thus present in most of the cases: shock and an oxytocic extract or drug. It is not improbable that the effect of this set of circumstances acting on a vasculature morbidly affected by pregnancy results in spasm of the small arteries and arterioles of the kidneys. The vascular disturbance is evident in the fact that the frequency of ablatio placentae is four times greater in cases of toxemia of pregnancy than in cases of apparently normal gravidity.[57] The spasm may be of sufficient intensity and duration to result in damage of the renal parenchyma, in some instances irreversible, in others transient, manifested by oliguria and uremia. In support of this hypothesis are reports of recovery, substantiated in 1 important instance by biopsy [58] of a woman's kidney after postpartum anuria developed. Figure 6*C* is the photomicrograph of the kidney of a rabbit killed forty-eight hours after intravenous inoculation of potent meningococcic filtrate, showing a small focus of typical cortical necrosis. The glomerulus and its convoluted tubules are necrotic. Similar small lesions are scattered throughout both renal

56. (*a*) Bowers, R. K.: Proc. Roy. Soc. Med. **27**:1505, 1934. (*b*) Geipel, P.: Arch. f. Gynäk. **124**:231, 1925. (*c*) Heist, J. C.: Am. J. Obst. & Gynec. **12**:673, 1926. (*d*) Herzog, G.: Beitr. z. path. Anat. u. z. allg. Path. **56**:175, 1913. (*e*) Immink, E. A.: Nederl. tijdschr. v. geneesk. **74**:2389, 1930. (*f*) Klotz, O.: Am. J. Obst. **58**:619, 1908. (*g*) Zooijer, G. H.: Mitt. a. d. Grenzgeb. d. Med. u. Chir. **12**:754, 1903. (*h*) Carson, W. J., and Rockwood, R.: Arch. Path. **1**:889, 1926. (*i*) Bruno, F. E.: New Orleans M. & S. J. **94**:596, 1942. (*j*) Jardine and Kennedy.[53h] (*k*) Westman.[53p] (*l*) Davidson and Turner.[53r] (*m*) Dunn and Montgomery[53s] (*n*) Kellar and Arnott.[53t] (*o*) Jardine and Teacher.[55]

57. Beck, A. C.: Obstetrical Practice, ed. 3, Baltimore, Williams & Wilkins Company, 1942, p. 706.

58. Crook, A.: Proc. Roy. Soc. Med. **20**:1249, 1927.

cortices, with normal-appearing tissue intervening. In appearance and distribution these lesions are identical with those illustrated in the drawing and photomicrographs of Crook's report.[58] In contrast, another animal in this group of 3 died twenty hours earlier, but its kidneys already showed the characteristic appearance of advanced cortical necrosis. The third succumbed six hours after the provocative injection, too soon for lesions to become visible. Such striking differences, also encountered by deNavasque in his experimental work, indicate that there is little reason to believe that in human as well as in experimental bilateral renal cortical necrosis an "all or none" law prevails, as some authors discussing reports of recovery would have their readers infer.

It appears from this discussion that in man numerous agents are capable of releasing a sequence of events leading to bilateral renal cortical necrosis. The most common cause is shock in parturient women. The same etiologic factor in males and nonpregnant females has been inculpated by numerous observers and confirmed to some extent by experiments utilizing known vasospastic substances.

An interpretation of the genesis of the renal lesion produced by both the generalized Shwartzman phenomenon and experimental meningococcemia based on the facts stated in the foregoing pages is as follows: The preparation of the kidney consists in the establishment of a state of vascular hyperirritability; the provocation, in a spasm of these vessels resulting in circulatory stasis, ischemia of the cortex and conglutination of the stagnant erythrocytes. Should the spasm continue beyond a certain time, necrosis of parenchyma and vessels occurs.

Several authors have reported the presence of varying degrees of necrosis of the liver, the adrenal glands, the spleen, the pituitary gland and the gastrointestinal tract in various combinations accompanying human renal cortical necrosis. Since the hypotheses examined in relation to the adrenal and renal lesions need no refurbishing, little is to be gained by an exhaustive review of these cases. It appears sufficient to record the fact that organs other than these two may respond to the same stimulus, shock or other, in a manner resulting in ischemic necrosis.

SUMMARY

Three experiments were carried out to investigate a possible relationship between the Shwartzman phenomenon and purpuric meningococcemia.

Experiment 1 served to confirm and elaborate the fact that twice-washed meningococci, both living and dead, possess potent preparatory and provocatory substances capable of producing the local Shwartzman phenomenon.

Experiment 2, by comparing the preparatory potency of 18 meningococcic strains, demonstrated that most of the strains (5 of 8) asso-

ciated with purpuric meningococcemia fall into a unique and very potent group. The strains obtained in cases of nonpurpuric meningitis produced less of the preparatory factor.

In serologic group distribution the two categories of meningococci were essentially identical.

Bilateral necrosis of the adrenal glands with hemorrhage was found in 2 animals of experiment 2.

Experiment 3 was designed to test the response of rabbits to meningococcemia maintained, if necessary, over a period of twenty-four hours. General cutaneous purpura was produced in a number of animals. In addition to the cutaneous lesions, 1 rabbit displayed marked adrenal necrosis and hemorrhage: Waterhouse-Friderichsen syndrome.

The close relationship of the general purpura to the local Shwartzman reaction was illustrated by the simultaneous appearance of both in rabbits which previous to their meningococcemia had been prepared in one or two sites by intradermal inoculation of meningococci.

Many of the animals of experiment 3 disclosed at autopsy bilateral renal cortical necrosis. Since in rabbits this lesion is recognized as characteristic of the generalized Shwartzman reaction, it is evident that washed meningococci are capable of producing not only the local but also the general phenomenon.

It is believed that the Shwartzman substance acts directly or indirectly on the interlobular arteries of the kidneys, causing marked vasoconstriction and thus initiating the sequence of events leading to bilateral renal cortical necrosis.

CONCLUSIONS

Washed meningococci, living or dead, are capable of producing the local Shwartzman phenomenon.

The organisms contain more of the potent substances than their supernatant fluids.

Meningococcic purpura has been produced in rabbits. The lesions are interpreted as a cutaneous manifestation of a generalized Shwartzman phenomenon.

The syndrome of Waterhouse and Friderichsen, which may complicate meningococcemia, has been reproduced in a rabbit. The adrenal lesions are considered to be a by-product of a general toxemia and not necessarily a resultant of the Shwartzman mechanism.

It is believed that the appearance of bilateral renal cortical necrosis in experimental meningococcemia is evidence of the ability of the washed bacteria to produce the general Shwartzman reaction. It is furthermore suggested that this lesion is effected not by thrombosis but by vasospasm with thrombosis as a sequel.

MARBLE BONE DISEASE

A Study of Osteogenesis

CARLA ZAWISCH, M.D.
PHILADELPHIA

THIS is not intended to be a presentation of a case of marble bone disease. It is an attempt to study a comparatively rare congenital bone disease from the moment of its onset in intrauterine life and through its further stages. It was felt that an ontologic study of congenital diseases may lead farther than the purely morphologic aspect and prove valuable for finding new therapies.

Marble bone disease is also commonly known as Albers-Schönberg disease, as osteopetrosis and as osteosclerosis fragilis generalisata. For the sake of simplification, however, the term "marble bone disease" will be used here. The chief histologic characteristics are: an extremely thick cortex of the long and short bones and a narrow marrow cavity filled to a great extent with medullary bone of abnormal structure. Some of the finer histologic details have been mentioned by various observers; others have up to now escaped attention. Roentgenograms show marked density of all bones, transverse and longitudinal bands of greater and lesser density, and an expansion of the ends of the long bones peculiar to this disease, the so-called clubbing.

The bibliography of marble bone disease up to 1934 has been collected in the excellent survey of McCune and Bradley.[1] Therefore the authors writing before 1934 will not be referred to in this paper except for special reasons. The same restriction will be observed in regard to the authors writing between 1934 and 1946 who did not furnish microscopic descriptions or whose roentgen reports do not contain any new detail.

The case which served for the present study was published by Windholz [2] during the lifetime of the infant, which was then 11 months old. The roentgenograms showed the typical marble bone changes in all the bones. The clubbing of the ends of the long bones was markedly developed for that age. There was severe atrophy of both optic nerves, but only slight anemia. No data could be obtained on the metabolism

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From the Laboratories of the Jewish Hospital.

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1. McCune, D. J., and Bradley, C.: Am. J. Dis. Child. **48**:949, 1934.
2. Windholz, F.: Ztschr. f. Kinderh. **51**:708, 1931.

of calcium and phosphorus. The father and the mother of the child were first cousins. The case must be classified among the malignant types (McPeak [3]) despite the fact that no fractures had been recorded in the still young infant; it belongs also to group I of Harnapp's [4] classification, i. e., malignant with probably recessive heredity.

The infant died about two months after it was examined by Windholz, and bone specimens became available for study. A series of cross sections were taken from a femur and a series of longitudinal sections from a rib. The sections were stained with Delafield's hematoxylin and eosin or with Heidenhain's azocarmine modification of the Mallory aniline blue connective tissue stain or were treated with tannin-silver after del Río Hortega's second method.

The cross sections through the middle part of the femoral diaphysis reveal the entire individual history of this bone. In normal development of bone four periods can be distinguished, starting with the first deposition of bone tissue in embryonic life. Each period is characterized by various types of bone tissue, which form an ascending line of differentiation. The timing of the periods naturally varies according to the time at which the various bones begin their ossification. In the femur (Zawisch [5]) ossification (not to be confused with the appearance of the calcification center of the cartilage on the forty-second day) starts in the ninth week with the appearance of a thin periosteal bone sheath or collar around the middle of the cartilaginous model (fig. 1 *A*). This is the narrowest part of the cartilage because here calcification started and prevented further expansion. From here calcification and also ossification spread toward the ends. During the first period, vessels from the periosteum grow into the cartilage and remove it by progressive resorption, while from the periosteum new layers of bone are deposited and form the first period cortical stratum. At the end of the first period (fourteen weeks, fig. 1 *B*), the cortex consists of various layers of early embryonic plexiform bone. It is thickest in the middle, where ossification began, and tapers out toward the ends. The primary marrow cavity is now formed. It is called primary because it results merely from the resorption of the cartilaginous model and has not yet been widened by resorption of the cortical tissue from within. Moreover, no medullary bone has been formed as yet. (The dark patches in figure 1 *B* are blood-filled sinuses.) From now on—i. e., beginning with the second period—the inner layers of the cortex are going to be resorbed from within in order to widen and to shape the (secondary) marrow cavity, while on the periosteal side the cortical strata of the subsequent periods are being deposited. Consequently, there must come a moment when the entire first period stratum as shown in figure 1 *B* has been removed and the marrow cavity is bordered by the second period stratum. This point is reached at about five and a half months of intrauterine life. In the end, all the embryonic strata are removed, and the marrow cavity is bordered by fourth period, i. e., lamellar bone. Medullary bone, or spongiosa, starts forming at the beginning of the second period. As all the processes which form and model the secondary

3. McPeak, C. N.: Am. J. Roentgenol. **36**:816, 1936.

4. Harnapp, G. O.: Monatschr. f. Kinderh. **69**:1, 1937.

5. Zawisch, C.: Ztschr. f. mikr.-anat. Forsch. **17**:41, 1929.

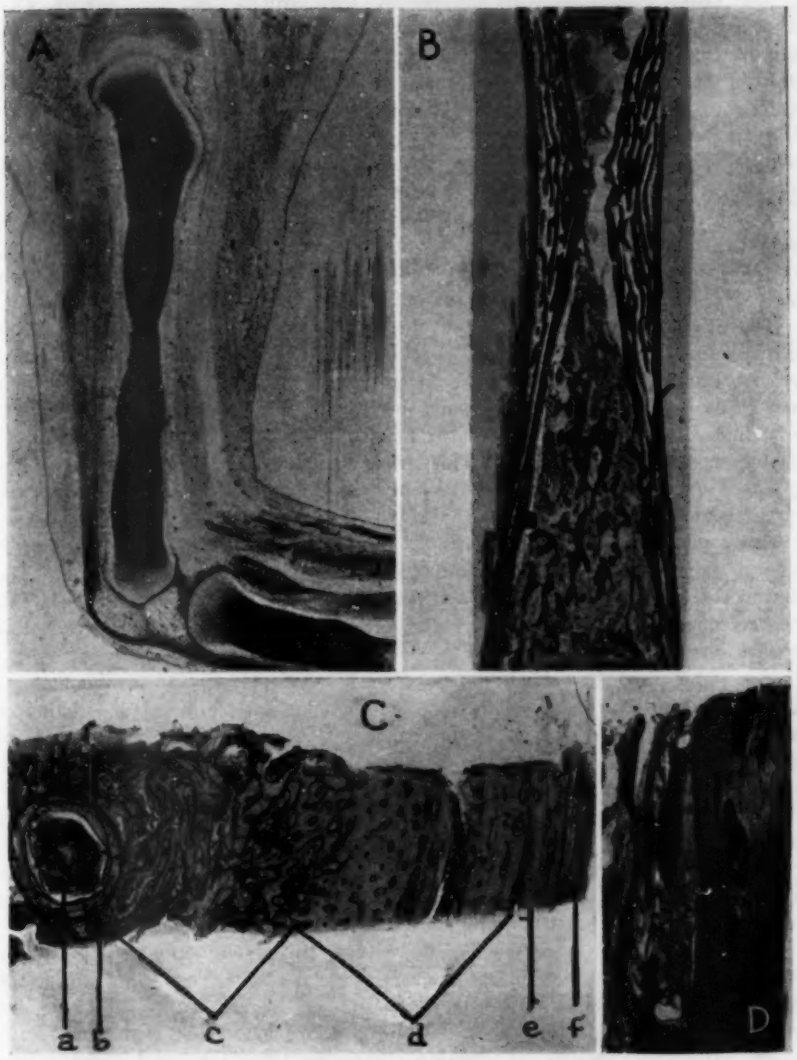

Fig. 1.—*A*, femur of an embryo at 8 to 9 weeks. × 12.5

B, femur of an embryo at 14 weeks. × 12.5.

C, sector of a cross section through a marble bone femur (middle of the shaft): (*a*) marrow cavity with trabeculae of the spongiosa and endosteal layers; (*b*) cement line marking the end of the first period; (*c*) second period stratum; (*d*) third period stratum; (*e*) periosteal stratum deposited during the fourth period; (*f*) periosteum. × 16.

D, longitudinal section of the diaphysis of a femur of an 11 month old normal infant for comparison with *C*. To the left are the marrow cavity and remnants of the otherwise resorbed third period stratum. To the right is the actual cortex, consisting of the fourth period stratum. × 32, reduced to × 16.

marrow cavity and the diaphysial cortex start at the middle of the diaphysis, cross sections through this region are the most revealing.

The cross section through the marble bone femur (fig. 1 *C*) shows what happened instead of the normal course of development. The diameter of the marrow cavity corresponds exactly to that of a fetus of 5½ months. It is bordered by a remarkably thick and deeply staining cement line, along which a few layers of endosteal bone have been deposited. This means that the first period stratum was removed normally but that afterward the widening of the marrow cavity was completely arrested. Resorption stopped at the unusually thick cement line between the first and the second period stratum.

The marrow cavity, moreover, contains a few spicules of spongiosa, whereas normally at this place there are none. Both these and the endosteal layers contain basophilic inclusions; i. e., they belong to a less differentiated type of bone tissue, not compatible with the actual age.

The present cortex consists of all the strata of the subsequent periods. This accounts for the unusual thickness of the cortex of marble bones. The strata are not equally arranged around the circumference; they are broader in some areas, narrower in others. The fourth period stratum (*e* in fig. 1 *C*) is particularly thin. It corresponds in size to that of an infant of 5 months instead of that of an infant of 13 months. This seems to indicate that the thickness of the cortex is due merely to lack of resorption and that growth and differentiation were retarded

For comparison, figure 1 *D* shows a section through the middle of a normal femoral diaphysis of about the same age and at the same magnification. The cortex consists merely of a thick fourth period stratum (pure lamellar bone). All the preceding strata have been removed, and only a few remnants protrude like spicules into the narrow cavity.

The strata in marble bones do not merely persist; they are also pathologic in their structure. The second period stratum (fig. 2 *A*), normally consisting of late embryonic plexiform bone, is mixed here to a great extent with chondroid bone. Chondroid bone of this type represents the lowest degree of differentiation in bone development. Normally it appears in the second period at the metaphysial ends of the cortex, where the bone grows in length. It plays a merely transitory role and is soon superseded by the ongrowing diaphysial cortex. Here it has been formed throughout the diaphysis and during all of the second period. Besides being out of place, it is also malformed. The most striking feature is the intense basophilia of its ground substance; i. e., the p_H is lowered.

The stratum which corresponds to the third period (fig. 2 *A* and *B*) is less severely malformed and in places appears even grossly normal. It consists of late embryonic plexiform bone, the interspaces of which are filled, or in the process of being filled, with prelamellar and lamellar bone belonging to the fourth period. However, patches of chondroid bone reappear here and there. This means that the bone-forming blastema had begun to recover in the direction of normal differentiation but that relapses occurred.

The stratum deposited during the fourth period (fig. 2 *B*) is normally differentiated and consists of lamellar bone, but it is poorly developed. In the part of the periphery shown in figure 2 *B* it is even thinner than that shown in figure 1 *C*, and in other parts it is missing altogether. The reversion to normal differentiation, although belated, is also shown by the fact that haversian systems and even rudiments of a mosaic structure have begun to form in the vascular spaces of all the strata, even within the chondroid bone of the second period stratum.

Fig. 2.—*A*, marble bone femur. It shows the second period stratum with adjacent parts of the marrow cavity (left) and the inner layers of the third period stratum. × 50.

B, marble bone femur showing the third period stratum with a large resorption space between the inner and the outer layer; also seen is a fourth period stratum with haversian lamellas. × 75.

In all the strata the unusually thick and deeply staining cement and junction lines are conspicuous. This phenomenon is likewise more pronounced in the inner strata and diminishes toward the periphery.

The various types of bone tissue forming the strata are pathologic in their finer histologic structure. Only the most striking of these details can be mentioned.

The osteoblasts are everywhere so flat that they are hardly distinguishable. In fact, they have escaped the attention of a number of observers, who have affirmed that there are none in marble bone disease. In this connection it must be mentioned that flat osteoblasts as such are not necessarily pathologic. Normally, the size and the shape of the osteoblasts depend on (*a*) the degree of the differentiation of tissue, and (*b*) the state of function (Zawisch [5]). The osteoblasts of the less differentiated types—chondroid and transition bone—are flat or spindle shaped originally, and the flattest osteoblasts are those which produce cement and junction lines. Cuboid osteoblasts are typical merely for the higher differentiated forms at the peak of function. They also become flat when exhausted. Therefore, flat osteoblasts may be considered as pathologic only if they are present in more highly differentiated types of bone tissue and throughout sizable areas. Otherwise they may indicate merely a regional ablastic period such as those which normally occur between productive periods. In the present case no other than flat osteoblasts could be found anywhere; therefore, they are certainly pathologic. There are also fewer than normal.

The osteocytes in chondroid bone are crowded, but in all other types of bone they are somewhat less numerous than normal. They also more often melt into ground substance. This can be seen comparatively easily in sections treated with tannin-silver. The same method shows that the canaliculi are longer and thinner than usual and that they form a denser network of anastomoses. This, according to Veit,[6] shows that the nutrition of the tissue is somewhat impaired. This is due to the relative paucity of osteocytes and the distance of central parts of the bone tissue from vessels after long periods of deposition. Therefore the network of canaliculi is stretched in order to make up for the deficiency.

In lamellar bone the individual lamellas are thinner than normal and densely packed. This is one of the reasons for the "density" of marble bone and explains why it is so hard to cut despite good decalcification.

Polarized light [7] reveals a relative scarcity of fibrils in all the bone tissues. Chondroid bone of the type which is hyperplastic in marble bone does not contain fibrils even normally, and the pathologic change in the ground substance consists here merely in an increase of basophilia. In the other bone tissues the scarcity of fibrils is very conspicuous. It diminishes with the recovery of the blastema, i. e., better differentiation, but is still marked even in lamellar bone. This scarcity of fibrils has a bearing on the calcium content of marble bones, as will be seen later. It is probably related to another phenomenon. There are indications that the substratum which furnishes the preosseous substance is less viscous than normal and precipitates more readily. But this leads into the domain of leptology where, as regards bone substance, little is known.

It may be mentioned, because it is sometimes contested in the literature, that osteoclasts are present in all stages of their evolution; budding, active and exhausted.

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6. Veit, O.: Inaug. Dissert., Freiburg i. Br., University Library, 1934.

7. Prof. G. B. Wislocki, head of the department of anatomy of Harvard Medical School, permitted me to use the polarization microscope of the department.

These finer details seem to indicate at least one thing: that all the various steps of the deposition process are taken at a stride and that what may be called the microrhythm is accelerated. A more detailed explanation of these phenomena cannot be given here.

The malformation of the bone is in itself responsible for the general retardation of resorption in marble bone disease. Resorption and deposition are two phases of one and the same bone-building process; they condition and stimulate each other by the biochemical changes which each phase produces (Zawisch [8]). If one of the phases is disturbed, the other will suffer likewise, and the entire process, in a vicious circle, becomes disrupted. This is evidently the case in marble bone disease. In normal bone, resorption occurs at a certain level of stimulation. In marble bone disease this threshold of response to the stimuli which bring about resorption is raised. In this femur the resorption of bone adjacent to the marrow cavity is completely arrested by the cement line deposited at the end of the first period. Internal resorption such as is normally found at the base of the internal reconstruction process is retarded. This retardation is due partly to the thickness of the cement and junction lines, which even normally act as a kind of temporary barrier against resorption, and partly to the biochemical properties of the pathologic bone tissues. Nevertheless, it follows even here certain general rules, one of which is that the longer a period of deposition lasts, the longer and the more active is the following period of resorption. Therefore, abnormally large resorption spaces are formed locally from time to time within the strata. (Compare *A* and *B* in figure 2.) Several such periods of resorption can be distinguished. The latest is the one that occurred in the second period stratum, where large, fresh and highly irregular resorption spaces can be seen. This stratum, the most pathologic, opposed the stiffest resistance to resorption. In the third period stratum, in a part of the periphery, not showing in figure 2, a phase of most energetic resorption occurred and came to a standstill some time ago. Large former absorption spaces are filled now with young, to a great extent lamellar, bone. These spaces, all of the same age, were confluent and irregular, so that during this period the stratum must have presented the aspect of osteoporosis. Indeed, temporary osteoporosis occurring in marble bone disease has been reported by several authors. Moreover, there is a large space between the outer and inner layers of the third period stratum (fig. 2*B*), filled with hemopoietic marrow. This space is present throughout the entire circumference at the level of the series of cross sections, and it is in open communication with other spaces containing blood-forming elements, whose joined surface area exceeds by far that of the marrow cavity at the level of the sections, so that they can be considered as an accessory marrow cavity.

8. Zawisch-Ossenitz, C.: Wien. klin. Wchnschr. **47**:801, 1934.

In the scanty fourth period stratum resorption appears normal, thus emphasizing once more the tendency to recovery.

Thus, the findings in the femur seem to indicate that marble bone disease starts at the beginning of the second period of bone formation. For the femur, this time is the middle of the fourth month of intrauterine life. By that time the thick cement layer had been laid down on the first period stratum, and from then on pathologic bone tissue was deposited. The first period stratum was probably normal, because it was normally resorbed. After this, further differentiation and growth were retarded. Resorption of the stratum from within did not take

Fig. 3.—Marble bone rib, part of a longitudinal section. Note the epiphysial line and the densely packed spongiosa. To the left, cortex and periosteum. Azan-Mallory stain; × 60.

place; therefore the cortex remained unusually thick. Internal resorption cannot make up for this failure and the entire bone-forming process remains disrupted.

The metaphysis always remains behind the diaphysis in development and differentiation. Therefore it can be expected to show the earlier phases of the development of marble bone still in existence and to furnish the clues for the development of the hyperplastic spongiosa. Figure 3 is a longitudinal section through the rib, taken from the chondroid-costal end. In the rib, ossification starts later in embryonic life than in the femur; therefore, the various stages are here even younger than in the femoral metaphysis.

The thin cortex of this part of the rib consists almost exclusively of second period chondroid bone of the same pathologic structure as that found in the femoral diaphysis. At the age of thirteen months it should be composed of

lamellar bone with remnants of chondroid and transition bone at the metaphysial edge around the cartilage. Therefore, the metaphysial cortex is still in its second period, the chondroid bone has never been resorbed, in order to make place for more highly differentiated tissues. Only slight attempts at further differentiation can be seen on the side of the periosteum.

The absorption front of the cartilage is normal, but the succeeding endochondral ossificataion is pathologic from the beginning. Normally the first period stage, in which no endochondral bone is formed, is being repeated here as long as the bone grows. The capillaries which absorb the cartilage always keep ahead of the osteoblasts, and deposition of bone begins at a certain distance from the absorption front. Thus the capillaries have time to absorb also a certain amount of cartilaginous ground substance. Here, as soon as a cartilage cell is liberated by absorption of its walls, basophilic matter and an osseous globule are deposited in the cavity (fig. 3, azocarmine method). Thus the cartilaginous ground substance is immediately sealed off against further absorption. Lying close to each other because no larger spaces have been created, the osseous globules coalesce almost immediately, and further deposition contributes to this process. The result is an extremely dense network of medullary bone which fills the entire marrow cavity.

This deposition of basophilic matter which in endochondral ossification reenforces the slender remnants of cartilaginous ground substance before real bone tissue is being deposited is also a characteristic of the second period formations. It is not of itself a pathologic formation, as some observers believe. It is the hyperplasia which is pathologic. The equivalent in the cortex is transition bone; therefore some observers mistake cortex for spongiosa.

Endosteal ossification in marble bone disease, therefore, shows the same pathologic development as cortical. A hyperplastic production of less differentiated bone tissue, containing more basophilic matter than usual, is at the root of the evil. That it also chokes off resorption is evident in this rib. Normally, waves of resorption, starting in the regions nearest the center of the bone and proceeding toward the ends, remove all superfluous spongiosa. They leave only such trabeculae as, after further differentiation, are being shaped into the typical trajectorial structures at the end of the shaft. Here no such resorption has taken place, and the marrow cavity is densely packed with spongiosa as far as the sections reach. The fact that even in the center of the shaft of the femur some spicules of medullary bone were found fits into this picture. Resorption of spongiosa, inhibited for a long time, never catches up completely.

A further tissue differentiation, corresponding to the age of the child, could not be found. In summary it can be said that the metaphysis of the rib shows the earlier stages of the process which could be retraced in the diaphysis of the femur and which accounts for the hyperplasia of medullary bone.

COMMENT

The literature in general seems to bear out the thesis advanced here that marble bone results from a specific second period disease of the

bone-forming blastema. Unfortunately, the spongiosa is more often described than the cortex, and no detailed description of the developing cortical bone can be found. A number of authors have maintained that marble bone disease is a malformation of "endochondral" bone only, meaning the spongiosa, and some writers have mistaken cortex for spongiosa because of the hyperplastic production of less differentiated bone tissues present in both. Others have expressed the belief that even these tissues—chondroid, osseous globules and basophilic inclusions —are pathologic of themselves. Therefore they are sometimes given special names which are hard to transcribe, whereas the proper terms exist and the pathologic changes consist in the hyperplasia and the malformation of these tissues. All this at times confuses the issue.

However, in the cases which have been reported since 1934 and in which microscopic observations have been described, the age ranging from birth to senescence, the findings corroborate the description given here and show fairly well the further evolution of marble bone disease.

The most thoroughly analyzed case is that observed by Dijkstra.[9] The patient was a child 3 years old. A rib, a vertebra and an unspecified piece of a cranial bone were examined. In the rib the state of the endosteal ossification was the same as in the Windholz case described here. But the cortical ossification was more advanced, the stratum consisting of late embryonic plexiform bone with a little lamellar bone. Dijkstra described it as *semblable à celui du fétus normal* (similar to that of a normal fetus), and indeed it corresponded to the third period, whereas in the normal rib of this age lamellar bone prevails. Differentiation had taken place but had not yet caught up.

The vertebra of Dijkstra's case is of considerable interest. It presented the characteristic double cone form of the primary marrow cavity which Dijkstra was first to describe for marble bone disease. The vertebrae begin ossification later than the long bones of the limbs. Dijkstra's specimen corresponded approximately to the femur presented here, only the double cone marrow cavity, pulled out by the great elongation of the femur, was clearly visible in the short vertebra. It was completely filled with spongiosa. The cortical stratum was formed by late embryonic plexiform bone with little lamellar bone. Thus differentiation was a little behind that of the rib, which corresponded to the ossification rates of these bones. Dijkstra's description of the marrow cavity induced Schmidt[10] two years later to suggest that the double cone form is at the basis of the well known clubbing of the ends

9. Dijkstra, O. H.: Ann. d'anat. path. **12**:131, 1935.

10. Schmidt, M. B., in Henke, O., and Lubarsch, O.: Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Julius Springer, 1937, vol. 9, pt. 3.

of long bones. However, other explanations for the clubbing are better substantiated, as will be shown.

Dijkstra showed an interesting feature in the bone of the calvaria. He distinguished three main periods of resorption in the normal development of the flat bones of the skull. The first concerns the inner table and models the curvature. In the second the plexiform bone is resorbed and replaced by lamellar. In the third the normal shape of the diploe is achieved by development of lacunas. In the case which he studied, the first period had developed normally, but the second and the third were greatly retarded and no diploe in the real sense was present. In the stratum corresponding to it, lamellar bone had already developed. In the cranial bones ossification starts earlier than in the vertebrae. The state of the marble bone changes in the calvaria corresponded to this situation. And, although a detailed analysis cannot be made here, the three stages of ossification in marble bone disease—normal beginning, malformation, repair—can be clearly distinguished in Dijkstra's description.

In Gerstel's [11] patient, a child 3½ years old, basophilic formations, probably even chondroid particles, were present even in the calvaria. This is not surprising, since they appear here also in normal ossification at very early stages. But they disappear soon. Their presence in a marble bone calvaria of this age shows that the process is particularly severe with respect to the bone changes. There are certainly various degrees of the bone malformation, and these degrees do not of themselves affect the fitness of the subject for survival. The rest of Gerstel's observations do not offer new details but provide a complicated nomenclature which is not possible to translate.

Clifton, Frank and Freeman [12] observed their patient almost from birth, and the child was 5½ years old when parts of the tibial metaphysis and of a rib near the costochondral junction were taken for biopsy. According to the size and the localization of the specimens, information is scanty. The cortex of the rib is still formed by chondroid bone; so differentiation in this case was particularly belated. The authors observed the basophilic inclusions and their character as neoformations with great accuracy. They stated, without arguing on the subject, that at some distance from the absorption front these formations were thicker than the slender cartilaginous remnants in the immediate vicinity. As such distinctions are not always easy to make in normal bone, it is pretty evident that the authors have seen the hyperplasia. In the tibial metaphysis the cortical tissue consists of late embryonic plexiform bone with chondroid inclusions. As differentiation of the tibial metaphysis normally begins later than that of the central parts of the bone and, on the

11. Gerstel, G.: Frankfurt. Ztschr. f. Path. **51**:23, 1937.

12. Clifton, W. M.; Frank, A. A., and Freeman, S.: Am. J. Dis. Child. **56**:1020, 1938.

other hand, is in advance of that of the metaphysis of the rib, this particular state of differentiation is just what one would expect in the marble bone tibia of a young child. But the absence of any differentiation in the rib suggests that the process was severer in this case than in the Windholz case with respect to the bone changes, and yet the child was alive at the age of 5½. Of the resorption process nothing is revealed besides the fact that no continuous marrow cavity is present.

The case of Lamb and Jackson [13] does not add any new facts. In the picture of the tibia of the 3½ year old child, the basophilic inclusions in the numerous and densely packed spongiosa trabecula are so large that their character as hyperplastic neoformations and the absence of any rebuilding process can be seen at the first glance.

The vertebra of van Creveld and Heybroek's [14] first patient, 9 days old, shows the same gross structure as that of Dijkstra's [9] patient; the authors only describe it the opposite way. Two wedges of bone (cortical) with the points directed toward each other "compress" the endochondral trabecula of the center. They expressed the belief that this cortex was normal but stated that the "cartilaginous rests" in the spongiosa were thicker in the center of the vertebra than at the periphery.

A small number of authors had an opportunity to study marble bones of adults. Laubmann [15] observed the disease in a young man of 25. He is one of those who, not having studied fetal bones, describe normal features as pathologic per se and apply to them a vague terminology which makes an analysis difficult. Essentially, Laubmann's case presents the same microscopic characteristics as those described by his predecessors. A picture of his vertebra shows rests of chondroid bone in the otherwise evolved cortex, which is remarkable in one of that age.

Heidger's [16] case was that of a man 58 years old. It is hard not to rule it out altogether as a case of marble bone disease. Certain details which the author described reminded him of rickets, and broad fresh deposits of primary bone substance (unfortunately called "osteoid" [17] by him) suggested to him that the aged man had also suffered from osteomalacia. Actually the patient died of bone abscesses and multiple myeloma. What remains of the marble bone features were remnants of what the author called "calcified cartilage" despite the fact that they were obviously larger than any real rests of cartilage ground substance

13. Lamb, F. H., and Jackson, R. L.: Am. J. Clin. Path. **8**:255, 1938.

14. van Creveld, S., and Heybroek, N. I.: Acta pædiat. **27**:462, 1940.

15. Laubmann, W.: Virchows Arch. f. path. Anat. **296**:343, 1935.

16. Heidger, P.: Beitr. z. path. Anat. u. z. allg. Path. **97**:509, 1936.

17. The tragic fate of the word "osteoid" becomes more and more obvious every year. Originally meant by Koelliker, in the 1880's, to designate the cellless bone of fish, it can be found in recent papers on bone disease for almost anything besides lamellar bone.

can be. But the author affirmed that they were "metaplastic neoformations of cartilage" because they were never found in desmal bones. This is confusing, for they are neoformation, but not of cartilage, and they do appear also in desmal bones in normal osteogenesis as well as in marble bone disease.

Heidger mentioned deeply staining cement and junction lines; Schmidt [10] described them also, as particularly thick. Heidger's case showed "eburnation," thus confirming preceding descriptions of the adult marble bone cortex and its "mosaic structure," i. e., a composition of relatively small and numerous haversian systems (osteons of modern terminology). Schmidt mentioned that the vessels are more numerous and more ramified than normal. That is precisely what causes the mosaic structure, because each osteon is formed around a vessel.

Thus the information on adult marble bones since 1934 is rather poor. But, pieced together with the information gathered before that time, it conveys a fairly good general impression of the process of repair. McCune and Bradley [1] enumerated the known patients whose cases have been reported, with respect to their ages, from the newborn infant up to the adult. It can be observed that in a general way the more advanced the age of the patient the more often the words "lamellar bone" and "haversian systems" occur in the description. Thus differentiation proceeds if the patient survives, but the mosaic structure reveals that during the period of interior reconstruction resorption and deposition continue in a pathologic rhythm and on a pathologic scale. The thickness of the cortex shows that resorption lags behind for a long time. Remnants of less differentiated bone tissues may or may not be present in the adult bones. In a single case, that of the 25 year old patient of Clairmont and Schintz (see McCune and Bradley [1]), the microscopic structure of the bone was described as "completely normal save for the increased branching of the haversian canals." But the material in this case consisted of a mere fragment obtained for biopsy from a not specified place in the femur.

In how far the finer details of the histologic structure mentioned on page 60 (fibrils, osteoblasts, osteocytes) also become more normal in the course of repair is hard to tell. They have not been investigated before, and I had no opportunity to study adult marble bones.

There is as yet no uniformity in the opinions of the various commentators as to whether marble bone disease is based primarily on a faulty bone-building process or on a primary lack of resorption. More precisely: Is it the deposition phase or the resorption phase which is at fault and, consequently, is it the bone-forming blastema or the vascular system which is diseased?

Most authors have confined their concepts to endochondral and endosteal ossification. McCune and Bradley [1] stated that the disturbance responsible for the sclerosis seems to be located in the endosteal and endochondral metabolism of bone: "The most widely accepted current concept holds that the fundamental defect in Albers-Schönberg's disease consists in a faulty differentiation and development of this primitive substance," i. e., the mesenchyma. Full credit for this clear formulation is due to the authors, because none of their predecessors had expressed it directly in this way.

In 1937 Schmidt [10] summarized his opinion and that of most authors by stating that Albers-Schönberg disease is the result of a lack of resorption. Nussey [18] one year later said that most authors have ascribed the departure from normal to a disturbance of endochondral formation. Dijkstra [9] decided for delayed resorption as the primary cause, and so have in recent years van Creveld and Heybroek.[14] Of the other writers mentioned here, most have been in favor of a primary disturbance of endochondral formation; only Gerstel [11] explained the disease by coining yet another word, *Dyssynusie,* which means a disturbance in the equilibrium of the tissues.

The modern concept of the bone-building process as a unit, the two phases of which—deposition and resorption—condition and regulate each other, tends to level out the discrepancies in the explanation of marble bone disease. Wherever deposition is abnormal, resorption is adversely influenced and vice versa. From the analysis presented here, it seems that the vascular system is not primarily affected, because resorption proceeds normally up to the moment when the blastema starts to function in a pathologic way, and then it becomes disrupted. In this sense, therefore, McCune and Bradley's formulation, that the fundamental defect is faulty differentiation and development of the mesenchyma, holds good and is much to the point.

The best observers, when venturing a conjecture, place the onset of the disease at an early stage of intrauterine development. It has been shown here that all the available data point to the second period of bone development as the time when the faulty differentiation begins. The question is whether the noxious influence acts during a certain period of intrauterine life and hits the bones which during that period are in a state of response, or whether it invariably attacks all the bones in the second period, regardless of the time at which the respective second period of each begins. In the first case, possible clues to the etiologic factor and the therapy might be found in the condition, hormonal or otherwise, of the mother. In the second case one is faced by the general problems of the "vitia primae formationis," of which in the special case

18. Nussey, A. M.: Arch. Dis. Childhood **13**:161, 1938.

of bone formation one does not know the first word. One is ignorant of the embryologic factors of induction and competence responsible for this process.

Unfortunately, investigators will probably have to decide for the second possibility. It is true that the clavicle, the bone first to begin ossification (at the start of the sixth week), has never been examined microscopically in cases of marble bone disease, and neither have the carpals, which begin latest (after birth), nor the epiphyses of the long bones. But the available roentgenograms show the clavicles to be diseased in the same way as the other bones, and they suggest this also for the epiphyses. This constitutes a wide range of time for the development of the disease. Therefore, one will have to conclude that it is a general failure of the fetal bone-forming blastema which becomes manifest whenever a bone enters its second period, i. e., when, at a certain level of differentiation, it is in a state of response to the noxious influence.

But as long as there are no histologic and developmental data concerning the bones and the epiphyses which ossify after birth, the last word in answer to this question remains to be said. It is to be hoped that in the next cases which come to the attention of observers, these bones will be examined likewise.

That the words "heredity" or "hereditary factor" present no real solution is clear. There is no doubt that many patients with marble bone disease show recessive heredity, but others do not. This merely proves that the real etiologic agent arises spontaneously in certain ones and may or may not become a transmissible character; it does not specify what this agent is. The word "heredity" is nothing but a shrug of resignation.

The roentgen picture of marble bone disease can be readily explained on the basis of the present microscopic observations. It comprises: the clubbing, the density of the bones in general and the bands of greater and lesser density within individual bones.

The clubbing of the ends of long and short bones has been explained by Schmidt [10] with reference to Dijkstra's [11] vertebra as resulting from the narrowing of the central part of the shaft. This is not quite to the point, because the shaft is not much thinner than normal, whereas the metaphyses are much thicker. This spreading out of the metaphyses gives the typical roentgenographic shadow known as clubbing and if the patient survives the epiphyses follow the broadened outlines.

The metaphysis is the place where normally the less differentiated types of bone are produced which provide for rapid growth. The amount always corresponds to the rate of growth. It is therefore greater in that end of a long bone which—as Ollier [19] had shown—grows more rapidly

19. Ollier, L.: Traité expérimental et clinique de la régénération des os, Paris, V. Masson & Fils, 1867.

than the other, e. g., the proximal end of the humerus or the distal end of the femur.

In marble bone disease, bone tissues of these types are hyperplastic. Consequently, as Cortes-Llado [20] has shown, the clubbing is more marked in those metaphyses which, according to the rules of Ollier,[19] grow more rapidly. These facts can be verified on all the roentgenograms published after 1934—e. g., in the papers of Shallow, Davis and Farrell,[21] Pounders,[22] Wortis,[23] Clifton, Frank and Freeman,[12] van Creveld and Heybroek [14] and especially Cortes-Llado.[20]

The phenomenon shows also in the case presented here. Figure 4 gives the outlines of the roentgenographic shadows of the femur and the humerus published by Windholz.[2] The clubbing is more marked

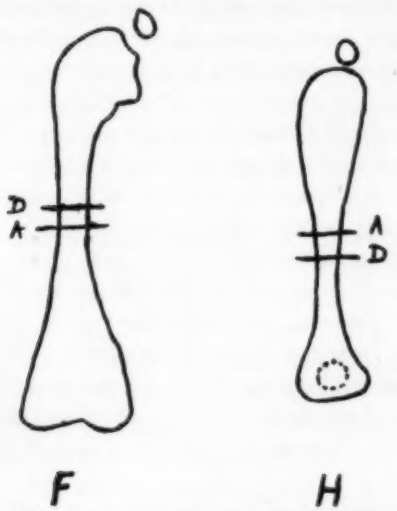

Fig. 4.—Outlines of the roentgenographic shadows of femur (*F*) and humerus (*H*) in marble bone disease: *A*, anatomic median line; *D*, developmental median line.

at the distal end of the femur and at the proximal end of the humerus. Moreover, another detail can be seen here and verified in the roentgenograms of other writers. The anatomic median line *A* divides the entire bone in two equal halves. The developmental median line *D* does so for the shaft alone and therefore marks the place where ossification started in fetal life. The developmental median line has receded toward the less rapidly growing end, i. e., toward the proximal metaphysis of

20. Cortes-Llado, A.: J. internat. de chir. **1**:63, 1936.

21. Shallow, T. A.; Davis, W. B., and Farrell, J. T., Jr.: Ann. Surg. **100**:512, 1934.

22. Pounders, C. M.: Ann. Int. Med. **8**:966, 1935.

23. Wortis, H.: Am. J. Dis. Child. **52**:1148, 1936.

the femur and the distal metaphysis of the humerus. In other words, the more rapidly growing ends are longer. That this difference in growth ratio is greater in these two halves than in normal bones has been clearly shown by Cortes-Llado [20] in a case which he observed during ten years. The author summarizes his observations by stating that the bones attacked by marble bone disease follow the rules of Ollier on a larger scale. They are subject to the same morphogenetic influences as normal ones but present the resulting phenomena in an exaggerated way. The difference in the growth ratio of the two ends is not the same in all bones. In figure 4 it can be seen that the difference between *A* and *D* is greater in the humerus than in the femur. This is also mentioned by Cortes-Llado for his case, and it can be seen in the roentgenograms of several other authors. An explanation of this special detail on the ground of normal development is not available at present.

The relative slenderness of the shaft of the long bones despite the thickness of the cortex is the result of the belated differentiation and its slow progress. This is not incompatible with the statement made previously that the microrhythm of deposition is accelerated, for this productive process spends itself with equal rapidity, whereupon an ablastic period follows.

The second characteristic of the roentgenogram is the "density" of the marble bone. Various components seem to be involved. Where lamellar bone is present, there is the density of the gross structure, i. e., the densely packed osteons (mosaic structure) and the dense microstructure of the individual osteon, i. e., the thin and closely packed lamellas. But this is evidently only part of the explanation. There is the problem of the calcium content of marble bones. That this is higher than normal has always been surmised but never definitely proved. Histochemical methods have not been used up to now, and the general ash contents have not been analyzed except by Clifton, Frank and Freeman,[12] who made use of a biopsy fragment for this purpose. They showed that the spongiosa has a higher ash content than the cortex.

The calcium and phosphorus metabolism test does not give any clues concerning the actual calcium content of the bones in marble bone disease. The results of the test are extremely variable, and the last decade has not brought forth any new evidence in this respect. Therefore, the opinion of McCune and Bradley [1] still holds good, that there is no way to determine, at present, whether it [the disturbance of the calcium and phosphorus metabolism test, if present] "is related directly to the course of the disease or whether it is simply an effect of the exaggerated production of bone." To this must be added that an abnormal outburst of resorption such as may occur in the course of the disease must needs mobilize a larger amount of calcium than usual. Therefore, it can be reasonably assumed that the variability of the results

of the test in cases of marble bone disease is due to the various states of the bones during the period of investigation. The results of the test must depend entirely on which phase of the exaggerated bone-forming process—deposition or resorption—prevails at that time.

The scarcity of fibrils in marble bones, however, reveals that the bones must indeed be richer in calcium than normal bones. The calcium salts are precipitated in the binding substance and not in the fibrils. If, as in the case of marble bones, the relative amount of binding substance is greater, these bones must contain more calcium than usual. Moreover, those types of tissue, e. g., chondroid bone and the basophilic inclusions, which even normally do not contain fibrils, are hyperplastic in marble bones, and this likewise must increase the absolute amount of calcium. In this way the results obtained by Clifton, Frank and Freeman,[12] indicating that the spongiosa has a higher ash content than the cortex, are explained; the spongiosa is richer in basophilic inclusions than the compacta.

The third characteristic of the roentgenogram is given by transverse and longitudinal bands of greater and lesser density. (See McCune and Bradley[1] and after 1934 Shallow, Davis and Farrell,[21] Root,[24] Schmidt,[10] Cortes-Llado,[20] Wortis,[23] Nussey,[18] Clifton, Frank and Freeman,[12] van Creveld and Heybroek[14] and others.) The more frequently seen are the transverse bands which run through the metaphysis of long bones parallel to the epiphysial line. In rarer cases longitudinal lines are found in the diaphysis parallel to the periosteum. Sometimes both types are present in one and the same bone. In flat bones such bands are seen as following the outlines.

It is clear that the transverse bands concern the spongiosa, the longitudinal bands the cortex. No microscopic evidence exists up to now to explain the former. McCune and Bradley[1] expressed the belief that the transverse stratification is the expression of the intensity of (*a*) longitudinal growth and (*b*) osteoblastic production and osteoclastic resorption. Cortes-Llado[20] said that it is the expression of the osteogenetic activity at the absorption front, which in fact means the same thing.

For the longitudinal bands present in the case discussed here, an explanation can be given. Windholz[2] called them *periostale Appositionsstreifen* (periosteal deposition bands). The word is badly chosen since it would imply that the depositing of bone under the periosteum is pathologic in itself, which of course it is not. What is meant is the presence of a subperiosteal zone of greater density which is apposed to an inner zone of lesser density. Now this corresponds exactly to the microscopic structure of the femoral cortex as shown in figure 1 *C* and

24. Root, J. H.: Am. J. Dis. Child. **49**:964, 1935.

2 *A* and *B*. The subperiosteal zone of greater density in the roentgenogram corresponds to the fourth period stratum plus the outer layers of the third period stratum. Then follows a zone containing large resorption spaces, as shown in figures 1 *C* and 2 *B*. This is bound to be expressed in the roentgenogram as a band of lesser density. Finally the inner strata must have appeared denser again. The resorption spaces in the innermost second period stratum appear so fresh that they probably were not present as yet when the roentgenogram was taken.

Therefore one can probably enlarge on the explanation of McCune and Bradley [1] and of Cortes-Llado [20] and say that the bands of greater and lesser density seen in the roentgenogram are brought about in two ways: (*a*) by periodic remissions and recrudescences of the pathologic deposition of bone and (*b*) by periodic recurrences of widespread resorption.

Such outbursts of resorption have been described miscroscopically by several authors (Schmidt [10]). That even osteoporosis occurs at times in marble bone disease was proved microscopically in the case of Kudrjawtzewa (McCune and Bradley [1]). This gives a clue to the apparently paradoxic fragility of marble bones. Fractures must occur more readily during a period of widespread resorption. Moreover, marble bones must be more brittle than normal bones because they are poorer in fibrils. Fractures in marble bones, for the most part, do not splinter, and the bones when pounded break up in the irregular fashion of glass, so that no definite planes of cleavage can be discerned (Clifton, Frank and Freeman [12]). This also points to the lack of fibrils.

It remains to be considered whether and in how far the anemia which generally accompanies marble bone disease can be explained by the developmental picture given here.

The question is linked in a way to that of the other forms of osteosclerosis combined with blood diseases, e. g., leukemia or pseudoleukemia. This part of the problem cannot be examined here. For the latter group of diseases too little histologic evidence is available, so that nothing is known about the relationship between the bone and the blood disease in these conditions. As Jordan and Scott [25] have put it, one cannot decide as yet for any one of four possibilities: The association is purely accidental, or osteosclerosis is the result of the blood disease, or the blood disease is the result of osteosclerosis, or the two processes occur at the same time and are due to the same etiologic agent.

Applied to marble bone disease, the two first possibilities can be ruled out. The third, that anemia is the result of the bone disease, has been understood up to now merely in the sense that a lack of space in the marrow cavity prevents the formation of a suitable amount of blood

25. Jordan, H. E., and Scott, J. K.: Arch. Path. **32**:895, 1941.

elements. As this explanation would cover merely the quantitative changes in the blood and not the qualitative, McCune and Bradley [1] expressed the current opinion by saying that only one assumption is possible and this is represented by the fourth alternative. Blood and bone have a common forerunner, the undifferentiated mesenchymal cell. The fundamental defect seems to be a faulty differentiation of the mesenchyma responsible for both. McCune and Bradley themselves expressed the belief that the faulty differentiation consists in the production of an excessive amount of abnormal osteogenetic tissue which proliferates at the expense of, or in place of, normal blood-forming elements. It would be, perhaps, simpler to surmise that the same agent which damages the bone-forming blastema at a certain level of differentiation affects equally the blood-forming blastema at a certain level. And this level may be where the hemocytoblast starts to differentiate in the direction of forming red blood cells.

However, modern histology has produced new evidence which is extremely suggestive and points once more in the direction of the third alternative, that the blood disease is the result of the bone disease. In various series of well planned experiments Roehlich [26] has shown that the production of marrow is strictly dependent on the resorption of bone. The veins of the cortical tissue open into the sinusoids of the marrow cavity. If this pathway is interrupted by whatever means, or if resorption does not take place, the production of bone-forming elements is arrested. It reappears immediately when resorption begins again and when the venous communications between the interior of the bone and the marrow cavity are reestablished. Roehlich supposed that by the resorption of bone a substance is set free which stimulates the production of marrow when it reaches the cavity by way of the venous channels. Such a substance was postulated by me [8] when I first described the self-regulating mechanism of bone deposition and resorption. The substance liberated by resorption can very well stimulate not only the formation of blood but also that of bone, and Roehlich's experiments offer corroborating evidence on this point too.

These results have a direct bearing on marble bones, in which defective resorption is most conspicuous. The vicious circle is inaugurated by faulty differentiation and by the production of a pathologic tissue resistant to resorption. The lack of resorption in its turn not only retards further differentiation of bone but also impairs hemopoiesis, because the necessary amount of stimulating substance is not produced. In this connection one may well remember the large resorption spaces filled with hemopoietic marrow within the cortex of the femur shown

26. Roehlich, K.: Ztschr. f. mikr.-anat. Forsch. **40**:425 and 616, 1941; **50**: 132, 1941.

here (figs. 1*C* and 2*B*). As soon as internal resorption breaks the pathologic barriers and enough bone substance is absorbed, marrow is produced even in places where it normally is not.

This concept would seem to show a way toward a therapy of marble bone disease. If the vicious circle could be broken by stimulating the resorption of bone, considerable improvement of the entire condition could be obtained in those patients who present a chance for survival.

SUMMARY

Marble bone disease is due to the influence of an unknown agent which damages the bone-forming blastema at the beginning of the second period of the development of each individual bone. From this time on, the differentiation of bone tissue is retarded and the less differentiated types become hyperplastic. The malformation is responsible for the general retardation of resorption, because the two phases of the bone-forming process—deposition and resorption—condition and stimulate each other. In marble bone disease the threshold of response to stimuli which bring about resorption is raised. Resorption is arrested at the beginning of the second period for a long time. The results are: fetal size and shape of the marrow cavity, a thick cortex composed of all the embryonic strata which normally are removed, and an unusual amount of spongiosa. The clubbing of the ends of long bones is the result of the hyperplasia and persistence of less differentiated types of bone tissue in the metaphyses. The greater density of marble bones in the roentgenogram is due partly to a greater density of structure when the bones are in a state of repair (eburnation) and partly to a higher calcium content, revealed indirectly by the scarcity of fibrils in all the types of bone tissue involved. The anemia in marble bone disease may be due to the same agent which impairs the differentiation of bone tissue. It is more likely that it is due to the relative lack of a substance which normally is released by the resorption of bone and stimulates the hemopoiesis.

EFFECTS OF ESTRADIOL BENZOATE AND THEIR MODIFICATION BY BLEEDING

Studies on the Skeleton and the Blood Calcium of the Cockerel

KURT SALOMON, M.D., Ph.D.*
BEVERLY WESCOTT GABRIO, B.A.*
EDWARD REINHARD, M.D.
AND
RUTH SILBERBERG, M.D.
ST. LOUIS

THE EFFECTS of estrogenic substances on bone do not always correspond to the changes in the serum or the plasma calcium level produced by these substances.[1] Therefore, other factors were thought to be involved in the skeletal response to estrogens. These factors may be of chemical or possibly hormonal nature; or local tissue changes, such as decreased vascularization or increased hyalinization of the bone marrow, may play a role in the condensation of the bones occurring under the influence of estrogen.[2] We thought, therefore, that an induced change in the activity of the marrow, such as hyperplasia after withdrawal of blood, might modify the skeletal effects of estrogens and thus provide some further insight into the mechanism of their action.

The present investigation was undertaken to test this assumption and also to determine whether the skeletal changes caused in cockerels by an estrogen can be correlated with the effects of the estrogen on the plasma calcium.

MATERIALS AND METHODS

Thirty New Hampshire Red cockerels of the same hatch and 30 days old at the beginning of the experiment were used. Each bird was kept in a separate cage and had access to food and water at all times. A starting mash was fed until the birds were 6 weeks old, and a growing mash was furnished from the sixth week until they were killed.[3] The birds were weighed at regular intervals.

*Now at the Department of Radiology, University of Rochester School of Medicine and Dentistry, Rochester, N. Y.

From the Biochemical Research Laboratory of the Departments of Neuropsychiatry and Radiology, and the Department of Internal Medicine, Washington University Medical School, and the Laboratory of the Jewish Hospital.

1. McLean, F. C., in Luck, J. M.: Annual Review of Physiology, Stanford University, Calif., Annual Reviews, Inc., 1943, vol. 5, p. 79. Pindborg, J. J.: Acta path. et microbiol. Scandinav. **22**:290, 1945.

2. (*a*) Silberberg, M., and Silberberg, R.: Arch. Path. **28**:340, 1939; (*b*) **32**:512, 1943. (*c*) Day, H. S., and Follis, H., Jr.: Endocrinology **28**:83, 1941. (*d*) Landauer, W., and Zondek, B.: Am. J. Path. **20**:179, 1944.

The cockerels were divided into four groups: (1) untreated controls (5 birds); (2) cockerels bled at intervals during the experimental period but not given further treatment (6 birds); (3) cockerels which at the end of a thirty day period of observations received injections of 0.12 mg. of estradiol benzoate [3a] daily for seven, nineteen or twenty-eight days, the injection being made into the pectoral muscle (8 birds); (4) cockerels bled as were those of group 2 and treated with estradiol benzoate as were those of group 3 (11 birds). Further details of the experimental arrangement are given in the table.

Blood was withdrawn by cardiac puncture as frequently as possible without producing severe anemia. For this reason the intervals between bleedings had to be increased in those birds which were bled for longer periods, in order to avoid the production of serious anemia. The hemoglobin was determined every two or three days by the Evelyn oxyhemoglobin method.[4]

The first determinations of plasma calcium were made at the end of the initial thirty day period of observation and before the first injections of the estrogen were given. Another series of blood calcium values was obtained immediately before the birds were killed. Each value given represents the mean of two or three determinations from samples of the same blood. A fraction of a drop of heparin was added to the blood to prevent clotting; otherwise sufficient amounts of serum could not have been obtained for analysis. The presence of such small amounts of heparin did not interfere with the accuracy of the calcium determination. Calcium was determined by a titrimetric method,[5] in which perchlorato ceric acid (the solution resulting from the dissolving of a cerium salt in perchloric acid) is used as the oxidizing agent of oxalic acid liberated from a calcium oxalate precipitate. Nitro-ortho-phenanthroline ferrous sulfate complex was used as the indicator. All determinations were made in plasma after ashing with a perchloric acid–nitric acid mixture. Test analyses had shown that the oxalate equivalent of 30 micrograms of calcium could be titrated with an error of plus or minus 0.2 per cent.

One day after the last injections had been given the treated animals and the nontreated controls were killed. The left femur was taken out and fixed in solution of formaldehyde U. S. P. diluted 1 to 10; it was then sawed into an upper and a lower part and decalcified in 5 per cent nitric acid; the pieces were then split lengthwise, the distal end in a sagittal, the proximal end in a frontal plane. Thus a full view of the epiphysis, the metaphysis and the diaphysis was obtained. The tissues were embedded in paraffin, sectioned and stained with hematoxylin and eosin.

OBSERVATIONS ON WEIGHT, HEMOGLOBIN AND PLASMA

Weight.—The initial and final weights can be seen from columns 4 and 5 of the table. The mean final weight of the nontreated controls (1 to 5) was 585 Gm. The mean final weight of 2 bled cockerels killed (19 and 20) four days subsequent

3. The Purina starting mash contains: dehydrated alfalfa meal, an oil containing vitamins A and D, yellow corn meal, meat scrap, oat middlings, Ribolac, mineralized salt, soybean oil meal, wheat bran, wheat germ, ground wheat, fish meal and dried whey.
The Purina growing mash contains: alfalfa meal, ground barley, steamed bone meal, calcium carbonate, an oil containing vitamins A and D, yellow corn meal, ground Kafir-Milo, fish meal, meat scrap, dried whey, Ribolac, mineralized salt, soybean oil meal, soybean meal special, ground wheat and fish solubles.
The chemical analysis and vitamin analysis of these mashes shows:

Protein, %	24.08	19.95	Phosphorus, %	0.95	0.66
Fat, %	3.43	3.92	Riboflavin, parts per million	5.00	4.60
Fiber, %	5.18	4.47			
Ash, %	11.44	6.41	Vitamin A equivalent, U. S. P. units per gram	8.00	5.60
Calcium, %	2.38	1.22			

3a. The alpha estradiol benzoate (Progynon B) was supplied by the Schering Corporation on the recommendation of Mr. L. H. Cramblet.

4. Evelyn, K. A.: J. Biol. Chem. **115**:63, 1936.

5. Salomon, K.; Gabrio, B. W., and Smith, G. F.: Arch. Biochem. **11**:433, 1946.

TABLE 1.—*Experiments on Cockerels*

	Number	Duration of Experiment, Days	Amount of Estradiol Benzoate Injected, Mg.	Weight, Gm.		Hemoglobin, Gm. per 100 Cc. of Blood		Total Amount of Blood Withdrawn, Cc.	Interval Between Last Bleeding and Death, Days	Plasma Calcium, Mg. per 100 Cc.		Skeletal Reaction	
				Initial	Final	Initial	Final			Initial	Final	Amount of Bone	Fibrosis
Control cockerels	1	37	....	197	505	7.6	...	....	..	10.26	10.21	+	0
	2	37	....	215	600	6.8	...	....	..	11.02	9.84	+	0
	3	49	....	190	550	7.8	...	....	..	13.45	13.77	+	0
	4	49	....	193	560	8.9	...	....	..	10.15	10.46	+	0
	5	58	....	219	705	7.9	7.9	....	..	8.91	10.21	+ (+)	(+)
Bled cockerels	19	37	....	210	495	9.5	6.3	73.0	4	10.21	11.02	+ (+)	(+)
	20	37	....	215	490	8.0	5.2	72.0	4	8.91	8.82	+	(+)
	21	49	....	208	550	7.8	6.5	80.0	14	9.72	13.77	+++	++
	23	58	....	194	530	7.0	6.9	82.5	23	11.50	11.66	+++	++
	90	58	....	197	540	8.6	5.5	83.5	10	10.26	8.91	++	+
	27	58	....	222	600	9.2	7.5	94.0	10	11.34	11.34	+ (+)	0
Estrogen-treated cockerels	7	37	0.84	178	510	8.0	...	....	..	7.94	9.88	+ (+)	(+)
	8	37	0.84	200	430	7.5	...	....	..	12.80	10.58	+	0
	9	37	0.84	203	450	7.1	...	....	..	10.37	12.64	+ (+)	(+)
	10	49	2.28	175	550	6.8	...	....	..	11.99	12.15	+++	++
	11	49	2.28	188	655	7.9	...	....	..	12.15	13.45	++	0
	14	58	3.36	164	585	8.2	7.7	....	..	11.34	8.10	++	++
	15	58	3.36	294	630	8.3	7.4	....	..	9.40	8.22	++	0
	17	58	3.36	177	625	7.9	6.1	....	..	10.04	10.21	++	+
Cockerels bled and treated with estrogen	40	37	0.84	188	475	7.8	4.9	78.0	4	11.56	13.93	(+)	0
	41	37	0.84	191	450	8.4	4.0	73.5	4	8.18	11.66	(+)	0
	29	49	2.28	188	505	8.8	7.6	76.5	14	8.91	13.28	+ (+)	(+)
	30	49	2.28	195	455	6.9	5.9	79.0	14	9.23	11.34	+ (+)	(+)
	31	49	2.28	197	480	9.8	4.6	80.0	14	9.58	9.00	+	0
	32	49	2.28	204	570	7.2	6.7	79.0	14	8.97	8.82	+++	+++
	34	58	3.36	220	710	8.8	7.5	82.0	23	9.56	10.53	++	++
	35	58	3.36	215	750	7.6	6.4	92.5	8	11.02	10.69	++++	+++
	37	58	3.36	226	620	7.9	7.3	88.5	8	9.88	10.69	++	+
	38	58	3.36	218	600	8.6	6.5	86.0	8	9.56	9.56	+++	+
	81	58	3.36	305	560	7.6	7.0	80.0	8	10.20	10.21	+ (+)	0

to the last bleeding was 492.5 Gm. The weights of the remaining cockerels bled ten days after the last withdrawal of blood and later did not differ from those of the nontreated controls. The mean final weight of the cockerels receiving the estrogen was 554 Gm., and that of the cockerels bled and treated with the estrogen, 561 Gm. The weights of the last two groups were thus only slightly lower than that of the nontreated controls (585 Gm.).

Hemoglobin.—The initial and final hemoglobin values are given in columns 6 and 7, and the total amount of blood withdrawn is shown in column 8. The fluctuation of the hemoglobin level varied in different birds. The maximum amount of blood which can be withdrawn from 30 day old cockerels without producing significant anemia had been determined by an earlier experiment on 10 birds, but in spite of this precaution anemia did develop in a few of the birds toward the end of the bleeding period. In no case was the anemia present for more than four to five days, and in the majority of the birds the hemoglobin level remained fairly constant throughout the experiment.

Plasma.—The initial and final values for calcium are given in columns 10 and 11. No intermediate determinations were made, since in a preliminary experiment on 10 cockerels of the same strain and age and treated in the same way the serum calcium was found to be constant at ten day intervals. In normal birds the plasma calcium fluctuated between 8.91 and 13.77 mg., with a mean of 10.83 mg.,, per hundred cubic centimeters; the calcium values of the bled birds ranged between 8.82 and 13.77 mg., with a mean of 10.62 mg., per hundred cubic centimeters. For birds treated with estradiol benzoate the calcium values were as follows: between 7.94 and 13.45 mg., with a mean of 10.7 mg., per hundred cubic centimeters for those receiving 0.84 mg. of estradiol benzoate, 12.4 mg. after administration of 2.28 mg., and 9.55 mg. after injection of 3.36 mg. of the estrogen. All these values are within normal limits, and there was apparently no correlation between the slight variations in the plasma calcium and the amount of estradiol benzoate injected; the highest value of calcium, 13.45 mg., was present after administration of 2.28 mg. of estradiol benzoate (no. 11), and a low value of calcium, 8.10 mg., was found after injection of 3.36 mg. of estradiol benzoate (no. 14). Similarly, birds which were bled and treated with this estrogen failed to show deviations from normal levels, the values ranging between 8.18 and 13.93 mg. per hundred cubic centimeters.

Distinct lipemia was observed in the plasma of all birds treated with 3.36 mg. of estradiol benzoate. It was manifested by an intense yellow color and a high viscosity, and the plasma exhibited a fatty layer on standing.

HISTOLOGIC OBSERVATIONS

Normal Cockerels (fig. *1 A*).—Three layers of cartilage could be distinguished in the growth zones: (1) the articular layer, composed of resting cartilage cells embedded in abundant eosinophilic matrix; (2) the proliferating layer, containing many more and larger cells, separated from one another by thin strips of basophilic ground substance and showing columnar arrangement; (3) the layer of vacuolated hypertrophic cartilage cells surrounded by small amounts of calcifying chondromucoid matrix. The hypertrophic cartilage cells were corroded by metaphysial capillaries, which often closely approached but actually did not enter the proliferating cartilage. They were accompanied by loose connective tissue and some osteoblasts. Between the advancing vessels, broad pegs of cartilage reached into the metaphysis and were eroded by the marrow. The cartilage was covered

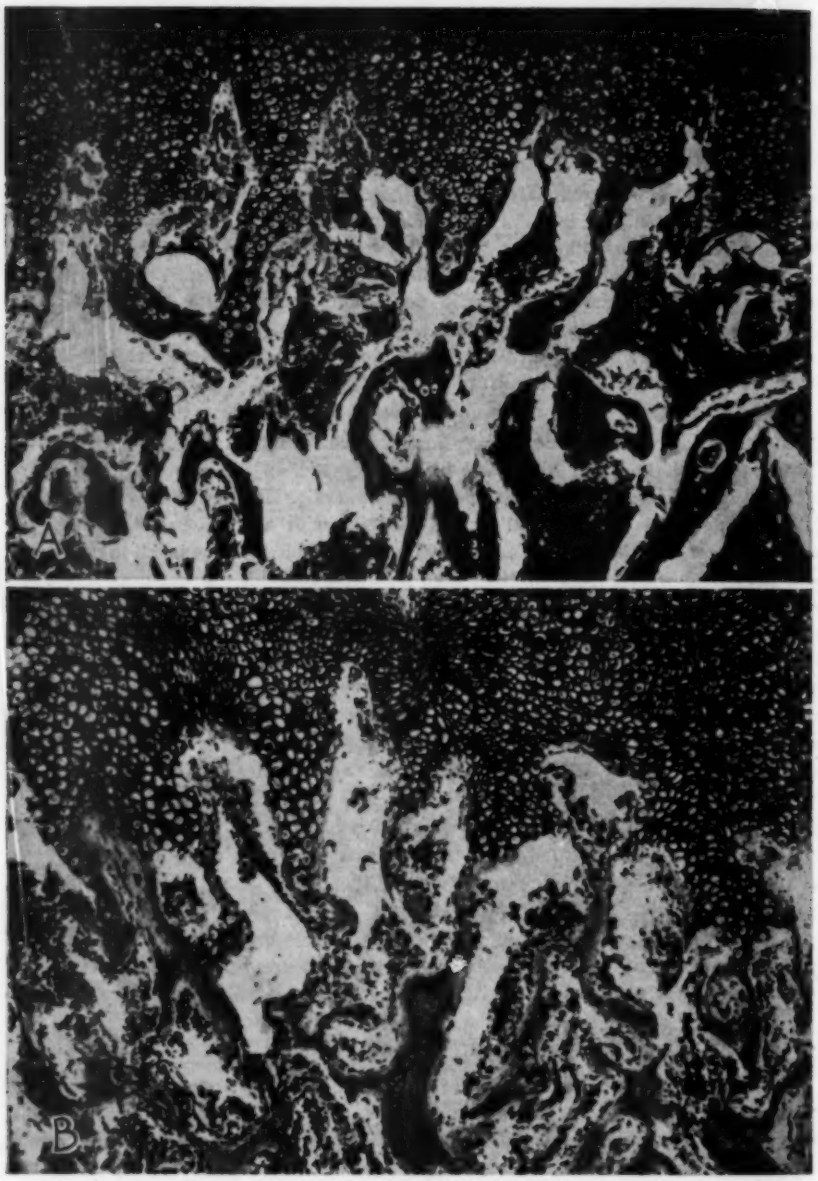

Fig. 1.—*A*, section of the epiphysis and the metaphysis of the upper end of the femur of an untreated 88 day old cockerel; × 50.5. Note the hypertrophic cartilage eroded by marrow and separated from the latter by a thin osseous lamella. The trabeculae are thin. The marrow is hemopoietic.

B, section of the epiphysis and the metaphysis of the upper end of the femur of an 88 day old cockerel which had been bled since the age of 30 days and killed ten days after the last bleeding; × 50.5. The bony spicules are more numerous and thicker than in *A* and are surrounded by a layer of fibrous tissue.

by a layer of bone, which was thin and discontinuous proximally but which increased in thickness farther distally. In the metaphysis, bony spicules were present, which here and there still contained unopened cartilage cells. These disappeared as the matrix between them calcified. More distally the spicules were covered by osteoblasts and surrounded by a narrow band of loose connective tissue. The marrow was otherwise hemopoietic. The shaft was composed of bone containing small osteocytes and vascular channels filled with loose connective tissue. Nearer the epiphysis the bone was less densely knitted and the channels were wider. The endosteum was discontinuous and thin. Just below the epiphysis the periosteum was thick; it extended into the bone and contained proliferating connective tissue with many multinucleated giant cells. Farther down, the shaft showed a broad transverse osseous protuberance reaching into the cavity.

Bled Cockerels (fig. 1 *B*).—At no time did the withdrawal of blood affect the cartilage. The number and the size of the cartilage cells and the consistency of the matrix were the same as in the normal controls. The condition of the bone and of the marrow of the metaphysis did not depend on the amount of blood withdrawn or on the length of the period during which the birds had been bled. There were, however, changes varying in degree with the length of the interval from the last bleeding to the time at which the cockerel was killed. In birds killed four days after the last bleeding, the metaphysial spicules were thin and long, and the marrow was congested and composed of hemopoietic tissue. In those killed ten days after the last bleeding the trabeculae, as well as the shaft, were thickened and dense and were surrounded by a layer of loose connective tissue. Both bone and fibrous tissue had increased in amount and density in birds killed fourteen and twenty-three days after the last withdrawal of blood. Farther distally in the diaphysis the marrow was congested and cellular. The shaft contained, besides large amounts of connective tissue, hemopoietic foci.

Cockerels Treated with Estradiol Benzoate (fig. 2).—The hypertrophic cartilage cells were decreased in number and size after seven days of treatment, and this effect was even more pronounced after nineteen and after twenty-eight days of treatment. The advance elements of the marrow consisted not of capillaries as usually but of a poorly vascularized connective tissue, with numerous osteoblasts often forming several continuous layers along the edge of the hypertrophic cartilage. The bony lamella deposited was thicker than that in normal animals and reached high up along the pegs of hypertrophic cartilage. The breakdown of the cartilage was less complete than usual. More cells were left uneroded and were incorporated in newly formed spicules, which thus were thicker than they would be normally and not infrequently interlaced with one another. The metaphysial stroma and that near the transverse protuberance became more abundant as the number of injections of the estrogen increased. Dense fibrous tissue surrounded the thickened and interlaced trabeculae. The fibers were arranged in bundles, new organic matrix appeared, and small preosseous spicules were formed. These increased in thickness and density farther distally, toward the diaphysis, took up calcium and thus were transformed into cancellous bone. The compact bone of the shaft became thicker and contained enlarged vascular channels filled with increasing amounts of connective tissue. The periosteum was likewise thickened and poorly vascularized, and there was increased deposition of bone. The endosteum was composed of a thick layer of connective tissue and numerous osteoblasts and was particularly abundant around the transverse protuberance. From here the newly formed fibro-osteoid tissue encroached on the metaphysis, linking up with the trabecular network therein. Toward the center of the diaphysial cavity these changes decreased, and hemopoietic marrow was present in all the birds.

Cockerels Bled and Subsequently Treated with the Estrogen (fig. 3).—The cartilage did not show any changes as compared with that of the nonbled birds receiving estradiol benzoate. After one week of injections the metaphysial spicules were numerous and long but thin. At the later stages there were many interlacing trabeculae showing cores of cartilage and surrounded by dense connective tissue. Moreover, bony substance formed apparently from the connective tissue. In the birds in which the process was most advanced, bone formation and myelo-

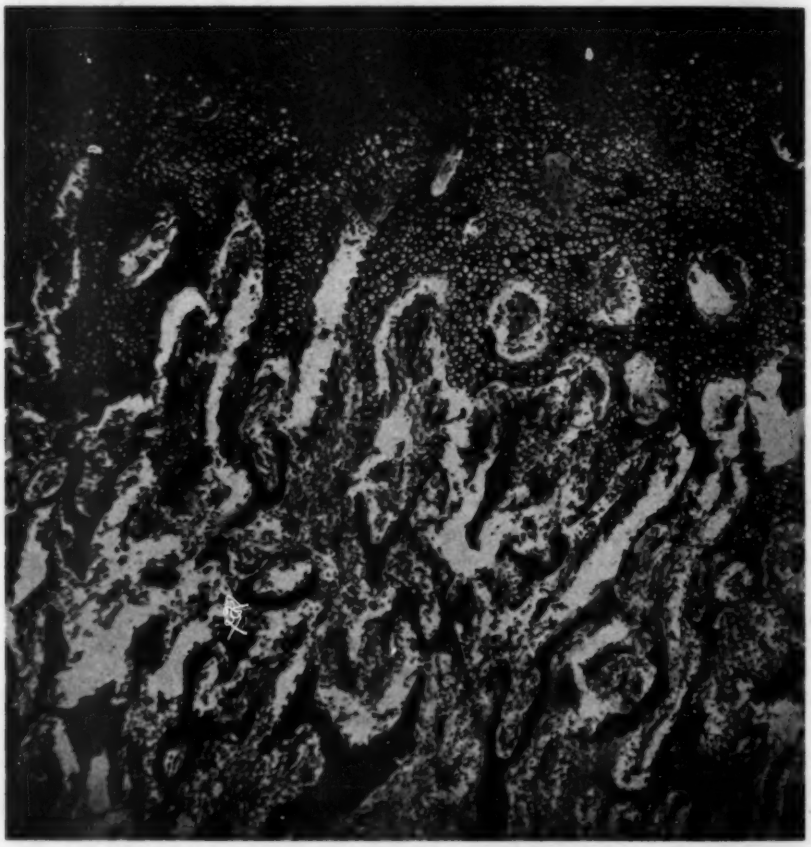

Fig. 2.—Section of the epiphysis and the metaphysis of the upper end of the femur of an 88 day old cockerel which had received 3.36 mg. of estradiol benzoate over a period of four weeks; × 52. Pegs of cartilage reach into the metaphysis. A moderate increase in the amount of bone and fibrous tissue is seen.

fibrosis exceeded those seen under the influence of the estrogen alone. However, there were islands of hemopoietic marrow scattered throughout the metaphysis. They were found even close to the hypertrophic cartilage crowded in between the layers of osteoblasts. The bone of the shaft contained small osteocytes and prominent cement lines. The vascular channels became wider as the bleeding and the injections of the estrogen were continued; but, whereas in nonbled cockerels treated with the estrogen the vascular channels contained only fibrous tissue,

there were many foci of hemopoiesis in the birds that had been bled prior to and during the estrogenic treatment. The endosteum and the periosteum resembled those seen in birds receiving the estrogen alone.

COMMENT

In normal, sexually immature cockerels, the plasma calcium ranged between 8.91 mg. and 13.77 mg. per hundred cubic centimeters. Repeated withdrawals of blood did not affect these levels. Cockerels of the same age

Fig. 3.—Section of the epiphysis and the metaphysis of the upper end of the femur of an 88 day old cockerel which had been bled since the age of 30 days and had received 3.36 mg. of estradiol benzoate over a period of four weeks and which was killed eight days after the last bleeding; × 52. Large amounts of cartilage are uneroded and incorporated into bony trabeculae. More bone and more fibrous tissue are seen than in figure 2.

receiving 0.84 to 3.36 mg. of estradiol benzoate over periods of from one to four weeks likewise showed plasma calcium levels within normal limits. In this respect the cockerels differed from male pigeons, which during the nonbreeding season and after castration showed an increase in serum cal-

cium following administration of an estrogen.[6] It is possible that an age factor operates in the reaction of the serum calcium to estrogens and that it is responsible for differences in immature birds, on one hand, and mature cockerels, on the other. As in the birds not given injections, bleeding did not change the serum calcium levels in the treated cockerels.

The results of the histologic examination can be summarized as follows: Withdrawal of blood did not affect the proliferation and the hypertrophy of the cartilage. In the metaphysis, however, there were increased fibrosis and formation of bone, increasing with lengthening of the interval between the last bleeding and the date of killing. In columns 12 and 13 of the table the degrees of the metaphysial and endosteal changes are demonstrated in a schematic way by the symbols + to + + + +, + for bone and 0 for fibrous tissue designating conditions found in untreated animals; + + indicates a slight, + + + a moderate and + + + + a striking reaction. There was no correlation between the histologic changes and either the hemoglobin or the calcium levels present at the time of autopsy; three almost identical hemoglobin contents of 6.3, 6.5 and 6.9 gm. (cockerels 19, 21 and 23) were associated with histologic reactions of low or moderate degree, respectively, and with a plasma calcium level of about 11 mg. per hundred cubic centimeters seen in three birds (19, 23, and 27), one moderate and two slight histologic reactions were recorded. The lack of correlation between the calcium levels and the skeletal changes seems especially interesting since in pigeons after the injection of estrogenic substances high calcium levels are due to elevation of the nonultrafiltrable calcium fraction.[7] The higher demand of the ossifying tissues for calcium may possibly be satisfied by increased turnover of the circulating calcium rather than by elevation of the blood calcium level. Moreover, the bleeding experiments support the view that local factors may be active in ossification and in the production of myelofibrosis.

Estrogen decreased the proliferation and the hypertrophy of the growing cartilage and the breaking down of the cartilage by the marrow, effects similar to those seen in small rodents after treatment with estrogen.[2a] Moreover, in the cockerels there were myelofibrosis and bone formation accompanied by increased resorptive processes.

The skeletal response to the administration of estradiol benzoate varied considerably in degree in different birds. Some, even after two weeks of treatment, showed hardly any response, whereas in others the reaction was impressive. The variations of the histologic picture were much more conspicuous than the slight fluctuations of the serum calcium level. The immature cockerel thus reacted differently from the mature

6. Bloom, W. A.; McLean, F. C., and Bloom, W.: Anat. Rec. **83**:99, 1942.
7. McDonald, M. R., and Riddle, O.: J. Biol. Chem. **159**:445, 1945.

cock, in which marked hypercalcemia and extensive hyperossification of the long bones have been observed.[8]

Our findings in immature cockerels are then, on the whole, comparable to those obtained in castrated pigeons and in male pigeons treated with estrogen during the season in which the testicles are resting.[6] It is thus possible that in the immature cockerel the absence of active testicles is responsible for the fact that the skeletal effects of estrogen are comparatively slight. However, the presence or the absence of active testicles does not fully explain the variations of the estrogenic effects in birds. Immature white Leghorn cockerels showed a more vigorous response to the administration of estrogen than old cocks and fully grown capons,[2c] a phenomenon also observed in mice [9] and rats.[2b] These variations again suggest the presence of an age factor which takes part in determining the reaction to estrogen. There is no synergistic action of androgen and estrogen in sparrows [10] and in certain strains of mice.[11]

Bleeding previous to and during the administration of estradiol benzoate did not alter the response of the cartilage to the estrogen. It did, however, modify the reaction of the metaphysial and diaphysial tissues in some of the animals treated for two or three weeks. There was no decrease in the myelofibrosis or the ossification as had been expected, but rather a tendency of these processes to increase beyond the degree seen after treatment with estrogen alone. The summation effect might have been even more pronounced, had the interval between the last bleeding and the killing been equally long in the groups receiving the estrogen and in the bled animals not receiving it. In the former the interval was only eight days, at which time the changes in the bled group had not yet reached their peak. The observations indicate that it is possible to modify the effect of estrogen by altering the substratum on which it acts.

SUMMARY

Injections of 0.84, 2.28 and 3.36 mg. of estradiol benzoate failed to raise the plasma calcium level of sexually immature cockerels above the normal upper limits of variation. The estrogen caused inhibition of growth and of breakdown of the epiphysial cartilage and produced myelofibrosis and hyperossification of the metaphyses and the areas close to the endosteal surfaces of the shaft. Cockerels that had been bled prior to and during the administration of the estrogen showed a tendency toward increased myelofibrosis and bone formation.

8. Landauer, W.; Pfeiffer, C. A.; Gardner, W. U., and Man, E. B.: Proc. Soc. Exper. Biol. & Med. **41**:80, 1939.

9. Silberberg, M., and Silberberg, R.: Anat. Rec. **80**:347, 1941.

10. Pfeiffer, C. A.: Anat. Rec. **94**:362, 1946.

11. Gardner, W. U., and Pfeiffer, C. A.: Proc. Soc. Exper. Biol. & Med. **38**: 599, 1938.

Case Reports

PRIMARY MYXOMA OF THE LEFT VENTRICLE WITH EMBOLIC OCCLUSION OF THE ABDOMINAL AORTA AND RENAL ARTERIES

ROBERT D. YOUNG, M.D., and WARREN C. HUNTER, M.D., PORTLAND, ORE.

PRIMARY tumor of the heart is sufficiently rare to warrant the recording of new instances, particularly those exhibiting important complications.

REPORT OF A CASE

M. S., a 10 year old white girl, entered the Portland Sanitarium and Hospital, Nov. 13, 1940, with the following history:

She had whooping cough in 1934 and measles in 1937. There were recurrent episodes of tonsillitis, with one severe attack in 1934. Pains first appeared in the joints in 1935 and have recurred yearly, especially during cold and damp weather. The pains were sometimes localized in the knees and at other times in the calves of the legs; they were not accompanied by swelling of the joints. The mother states that the first knowledge of any heart trouble came in 1936.

The present illness began one week prior to the patient's entering the hospital, when there was complaint of aching in the muscles of the calves. November 11 she awakened at night with vomiting and diarrhea, complained of pains in the legs and had difficulty in walking. On getting up her legs seemed to cave beneath her. During the afternoon of November 12, convulsions occurred at frequent intervals, and her temperature rose to 101 F. November 13, the convulsive seizures were still occurring, and the patient became completely stuporous. A typical seizure began with slight trembling jerkiness of the left wrist, while the eyes deviated to the right and a coarse nystagmus was directed to the left. After fifteen to twenty seconds this shifted, and similar jerkiness was noted in the right wrist, a little larger and more extensive than that in the left, together with conjugate deviation of the eyes to the left and coarse nystagmic jerks to the right. Later there were some jerking movements of the whole right arm and leg. The entire episode lasted for several minutes.

The child was well developed and well nourished and in a completely stuporous condition. The skin was fair and without evidence of petechial hemorrhages. There was no stiffness of the neck and no facial asymmetry. The conjunctivas were clear; the pupils measured 4.5 mm. and were round and equal; the response to light was prompt and adequate. The optic disk showed no abnormality; the physiologic cup was well defined; the vessels were normal; there were no hemorrhages. The tonsils were absent; the pharynx was boggy and reddened. The tympanic membranes exhibited a little diffuse redness but no bulging.

The tonus of the arms and the legs was normal, with movement possible in both arms and legs. The deep reflexes of the arms and the legs were active; there was a bilateral positive Babinski reflex; Kernig's sign was not shown.

The lungs were clear to auscultation and percussion.

Examination of the heart revealed sinus tachycardia, with a systolic thrill at the apex. The heart was slightly enlarged to the left without filling of the waist.

From the Department of Pathology, University of Oregon Medical School.

There was a 1 to 2 plus lower sternal thrust. The mitral first sound was followed by a rough systolic murmur, transmitted well into the axilla and up over the precordium. There was a loud systolic murmur at the aortic area, different in character from the mitral systolic. The aortic second sound was well heard, not accentuated and louder than the pulmonary second sound; it was followed by a definite soft diastolic blow, which could also be heard at the third left interspace and was transmitted down to the left sternal border.

The abdomen was not tender and was without masses and without palpable organs.

The impression was old rheumatic heart disease, acute pharyngitis with gastroenteritis, and serous meningitis.

The spinal fluid pressure was grossly increased; the fluid was clear. The total protein content was 47 mg. per hundred cubic centimeters; the sugar content, 87 mg.; the chloride content, 759 mg., and the cell count, 0.

The blood showed 26,000 leukocytes per cubic millimeter, 82 per cent of which were polymorphonuclear leukocytes and 8 per cent staff cells. The red cell sedimentation rate was 15 mm. in forty-five minutes.

As the day (November 13) advanced the clinical picture changed considerably. The convulsive seizures stopped after the administration of small doses of phenobarbital; marked facial edema developed; no urine was passed over a twenty-four hour period, and catheterization yielded only a few cubic centimeters of very bloody urine with some pus. The blood pressure was 150 systolic and 84 diastolic. No petechiae appeared anywhere.

The clinical impression then was: acute rheumatic fever; acute glomerulonephritis with anuria; old rheumatic heart disease with mitral insufficiency (and probably stenosis); aortic insufficiency and stenosis. It was not thought at that time that subacute bacterial or acute bacterial endocarditis was present.

Under continued sedation the convulsions did not recur; the almost complete anuria continued, as did the facial edema. The blood urea level was first 107 mg. and then, later, 170 mg. per hundred cubic centimeters; the systolic blood pressure climbed to 170 mm. of mercury. A second study of the blood gave 35,000 leukocytes, with polymorphonuclears 82 per cent and staff cells 8 per cent; the sedimentation rate was 11 mm. in sixty-seven minutes.

The impression was the same as previously, although now embolism with blocking of the renal arteries and renal infarction was considered.

On the last day of life a new phenomenon developed. Following the long apical systolic sound there was a loud snap, "like a shot," as if the valves had suddenly ruptured. This came not at the time of the mitral first sound but after the mitral systolic murmur. It would last for a few beats and then disappear. The sound was so loud that it could be heard several inches away from the chest. In view of the lack of constancy and timing the sound was not believed to be derived from the heart but to be rather some anomalous extracardiac sound. The child died on November 18, five days after admission.

The final diagnosis was: old and active recurrent rheumatic fever; old rheumatic heart disease with aortic stenosis and insufficiency, mitral insufficiency and probably stenosis; acute fulminating glomerulonephritis with uremia.

Autopsy.—Permission to remove the brain was denied, and for this reason the cause of the convulsions, if located in the brain, remains obscure. Since the only pathologic processes were confined to the cardiovascular system and the kidneys, only the positive findings need be given.

(*a*) Heart: This organ had a transverse diameter of 11 cm. Aside from pericardial and subepicardial petechial hemorrhages the surface of the heart was unchanged. The left ventricle formed the entire apex, and its left border was prominent and rounded. The right side of the heart, together with the great vessels entering and leaving it, was unchanged. The foramen ovale was closed. The wall of the left ventricle measured 2 to 2.5 cm. in thickness, and its muscle was firm and of coarse texture. The anterior leaflet of the mitral valve appeared thickened and whitened along the line of closure but was without active vegetations; the chordae tendineae of this valve were thickened but not appreciably shortened. While stenosis was absent, it was considered possible that an insufficiency may have existed during life. The left atrial appendage was free from thrombosis. While all cusps of the aortic valve were thick enough to be white and opaque, there was no apparent stenosis. The left posterior leaflet exhibited two small fenestrations near one of its commissures. No vegetations were present. Just above the aortic valve were three transversely directed atheromatous streaks involving the aortic intima.

Practically filling the left ventricle and extending upward to the level of the commissure of the aortic valve were multiple polypoid masses, the largest of which measured 5 by 3 by 2 cm. (fig. 1, at the left). This and others of smaller size appeared to be intimately attached to the endocardium over an area of approximately 1.5 to 2 cm., at a point a little below the insertion of the posterior papillary muscle of the mitral valve. Much of the main mass was jelly-like, smooth of surface and faintly yellow, although at the base the color darkened and the texture became firmer. A second soft smooth-surfaced yellow polyp, measuring 2.5 by 1 by 0.8 cm., was attached by a pedicle 3 mm. in diameter; a third, of similar appearance, measured 2.5 by 1.5 by 1.5 cm.; a fourth, nestled in the apex of the ventricle, had dimensions of 2 by 2 by 1.5 cm. and differed from all the others in being dark red throughout. All polyps appeared to have a common attachment along with the major polyp extending through the orifice of the aortic valve. However, a sixth mass, measuring 2 by 0.5 by 0.5 cm., sprang from the endocardium at the level of the free margin of the posterior leaflet of the mitral valve. Finally, on the left border of the base of the largest polyp and very near the pedicles of two others, was a slightly elevated, roughened surface, 5 mm. in diameter, which appeared to represent the point from which something had broken off. No thrombi occurred over the roughened area or anywhere else in the left ventricle.

(*b*) Abdominal Aorta and Kidneys: Lodged in the aorta at a point slightly caudal to the ostium of the celiac artery and extending distal to the ostiums of the renal arteries was a shiny-surfaced, soft and wet-appearing mass measuring 5.3 by 1.3 by 1.5 cm. (fig. 1, at the right). The distal end was somewhat pointed, whereas the proximal end was rough, ragged and apparently torn. The embolus was lightly attached to the aortic intima at a number of points, and when it was removed the vessel's surface at these places was slightly roughened. The mass was spongy, yellow, wet, and bore a close resemblance to the polyps in the heart. There was a little space between the embolus and the ostium of the superior mesenteric artery.

The main renal arteries were patent near the kidneys, but next to the aorta they were occluded by very soft pale masses directly attached to the mass filling the aorta. The kidneys, after the renal capsules had been stripped off, proved to be mottled, being alternately yellowish white to brown to black. When they were sectioned, extensive anemic infarction was seen to involve the cortex, often extending into the columns of Bertini. By contrast, the pyramids, especially those of

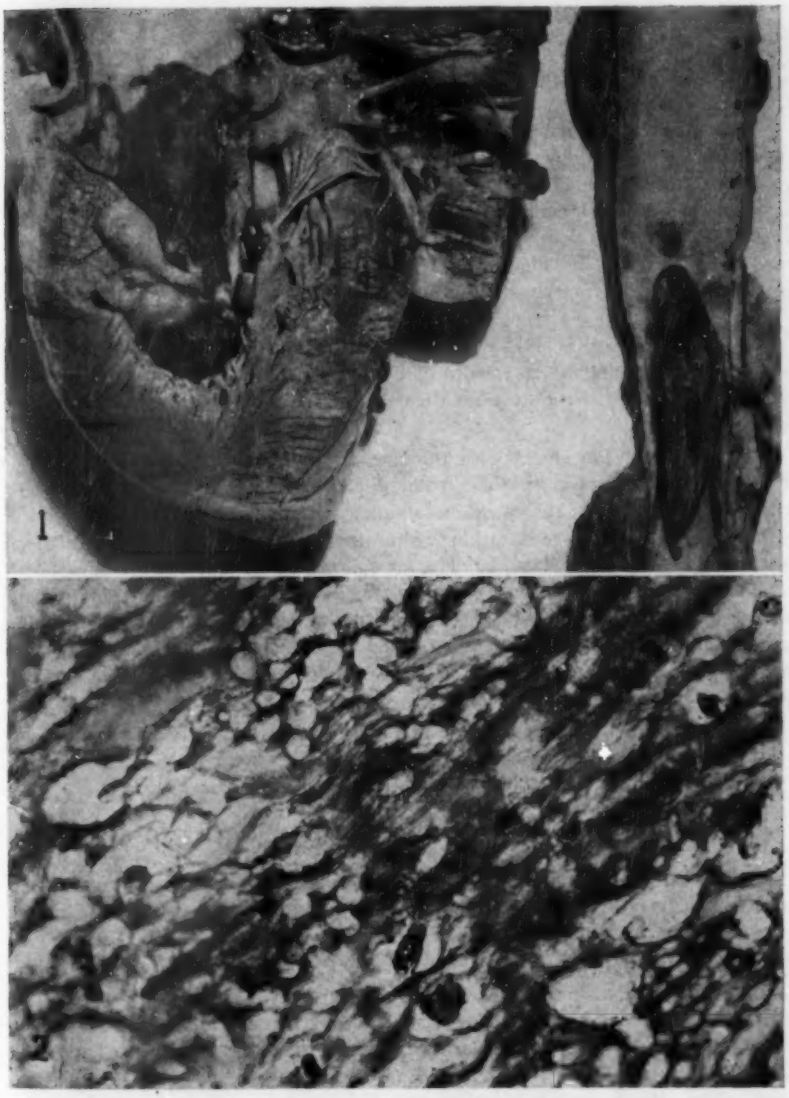

Fig. 1.—Visible along the septal aspect of the left ventricle are five smooth-surfaced polypoid masses having a common origin near the insertion of the posterior papillary muscle of the mitral valve. The largest projects through the aortic valve, the cusps of which are scarred as a result of healed rheumatic valvulitis.

Lodged in the abdominal aorta is a large tumor embolus which, being soft, extruded into the renal arteries, with resultant bilateral renal infarction and acute renal insufficiency.

Fig. 2.—High power photomicrograph illustrating the cell-poor structure of the tumors. Both the homogeneous and the more vacuolated portions give the staining reaction of mucin.

the left kidney, were almost black. Numerous petechial and ecchymotic hemorrhages involved the renal pelves.

(*c*) Lungs: These showed hyperemia, focal atelectasis and edema.

Microscopic Examination.—Sections from different parts of the main polypoid mass and sections of the dark red polyp, as well as some including the point of attachment of these bodies, revealed a structure poor in cells and consisting mostly of a homogeneous or stringy substance staining all the way from blue through bluish pink to red with hematoxylin-eosin. In the stringy substance were many crevices or larger openings, often bordered by ovoid or rounded cells of considerable size with deeply staining nuclei. The different polyps appeared to be well anchored to the heart wall by capillary vessels, by the homogeneous substance already described and by fibroblasts. The only additional cells within the polyps were neutrophilic polymorphonuclear leukocytes and monocytes except in the very dark tumor, in which the color was explained by the presence of a large hemorrhage. Endothelial cells could not be demonstrated over the external surface of any of the tumors, despite its smooth and shiny appearance. Despite the apparent lack of endocardium, there were no thrombic deposits anywhere over the various polyps.

Sections stained for mucin with thionine displayed varying shades of pink to red in much of the wavy bandlike material and the other more homogeneous or vacuolated intercellular substance, a staining reaction compatible with mucin. The nuclei of all cells were deep blue, while the cytoplasm of all known cells was either faintly blue or unstained. The cytoplasm of certain rather infrequently encountered cells with ovoid to elongated nuclei, containing scattered chromatin granules, appeared pink to red and extended out in a stringy and pointed fashion, often on only one side of the nucleus. Thus the cytoplasm of the cells just mentioned may be said to have had the same staining reaction as the intercellular substance, which was that of mucin.

The structure of the aortic embolus and of the extensions into the renal arteries corresponded to that of the tumors in the heart.

There was no microscopic evidence of active rheumatic endocarditis or myocarditis.

COMMENT

A summarization of the literature dealing with primary cardiac tumors is not required at this time. The subject has been well covered in the reviews of Lisa, Hirschhorn and Hart [1] and Yater.[2]

The unusual features of our case were (1) the left ventricular origin of the tumor and (2) the embolic occlusion of the abdominal aorta and renal arteries by a large tumor embolus. Embolic phenomena associated with these relatively friable growths are surprisingly rare and when present are most often found in the cerebral arteries. Often, as in our case, there occur relative stenosis and insufficiency of a valve due to the fact that the pedunculated tumor mass projects through the valvular orifice. A left ventricular origin of myxoma, while infrequent, nevertheless stands next in incidence to a left atrial origin. The question arises as to whether the tumor was true myxoma or an organ-

1. Lisa, J. R.; Hirschhorn, L., and Hart, C. A.: Arch. Int. Med. **67**:91, 1941.
2. Yater, W. M.: Arch. Int. Med. **48**:626, 1931.

izing thrombus. On the basis of the multiple polypoid character and the staining reaction with thionine, the weight of evidence is in favor of myxoma.

SUMMARY

An unusual multipolypoid primary tumor diagnosed as myxoma originated at the apex of the left ventricle. As a result of embolization of the abdominal aorta there were first the symptoms of acute intestinal obstruction. These were followed by bilateral renal infarction and fatal acute renal insufficiency. The temporary character of the symptoms of intestinal obstruction is explained by the soft and compressible character of the embolus, which allowed it to sink downward, freeing the ostium of the superior mesenteric artery. The same qualities allowed the embolus to extrude into the ostiums of the renal arteries. The initial episode of convulsions, stupor and nystagmus may have been due to cerebral embolism but denial of the privilege of removing the brain leaves this phase of the clinical course unexplained. The striking change in the heart sounds during the latter part of the child's life may have coincided with the breaking off of the tumor embolus.

PULMONARY ADENOMATOSIS COMPLICATED BY LOBAR PNEUMONIA

CARTER M. ALEXANDER, M.D., BINGHAMTON, N. Y.
and
FOO CHU, M.D., NEW YORK

PRIMARY multiple tumors of the lungs composed of epithelial cells lining pulmonary alveoli and without extrapulmonary metastases are rare. At least 9 cases have been recorded in the literature. Arranged in chronologic order, they are the cases of Helly [1] (1907), Oberndorfer,[2] Bonne,[3] Richardson,[4] Sims,[5] Bell,[6] Taft and Nickerson [7] (2 cases) and Wood and Pierson.[8] Other cases which may possibly belong in the same group have been reviewed by Neubuerger and Geever [9] in a discussion of alveolar cell tumors.

Because of the rarity of this neoplasm, we wish to report another case, in which there was terminal lobar pneumonia.

REPORT OF A CASE

J. H., a 56 year old white widow, was first admitted to the Second Medical Division of Bellevue Hospital on May 17, 1940, with the chief complaint of weakness of the right side of the body for twenty-four hours. The Wassermann test of the blood and the spinal fluid showed a 4 plus reaction in each instance. A roentgenogram of the chest disclosed a "fibrotic patch in the second right interspace in the axillary line." There was a rapid return of function in the extremities following therapy with malaria and oxophenarsine hydrochloride. During her follow-up in the clinic, the results of tests of the blood and the spinal fluid reverted toward normal. She was discharged July 15, 1940.

From Jan. 8 to May 1, 1945 the patient was again in the hospital because of fever, pain in the right anterior part of the chest and bloody sputum. Four months prior to admission she noted the onset of localized wheezing in the right upper part of the chest and had a cough productive of scant mucoid sputum. Two weeks before admission she "caught a cold," which was followed by fever, pleuritic pain, cough and increased sputum.

From the Department of Pathology, New York University College of Medicine and Bellevue Hospital, and the Second Medical Division, Bellevue Hospital.

1. Helly, K.: Ztschr. f. Heilk. **8**:105, 1907.
2. Oberndorfer, S.: Virchows Arch. f. path. Anat. **275**:728, 1929.
3. Bonne, C.: Am. J. Cancer **35**:491, 1939.
4. Richardson, G. O.: J. Path. & Bact. **51**:297, 1940.
5. Sims, J. L.: Arch. Int. Med. **71**:403, 1943.
6. Bell, E. T.: Am. J. Path. **19**:901, 1943.
7. Taft, E. B., and Nickerson, D. A.: Am. J. Path. **20**:395, 1944.
8. Wood, D. A., and Pierson, P. H.: Am. Rev. Tuberc. **51**:205, 1945.
9. Neubuerger, K. T., and Geever, E. F.: Arch. Path. **33**:551, 1942.

She was acutely ill, moderately dyspneic and slightly cyanotic. The pupils were equal and reacted to light. The trachea was deviated to the right. There were signs of consolidation over the upper lobe of the right lung.

The hemoglobin content was 13.5 Gm.; the white blood cell count, 18,000 per cubic millimeter, with 90 per cent polymorphonuclear leukocytes and 10 per cent lymphocytes The urine was normal. The Wassermann test of the blood was negative. Repeated examinations of the sputum revealed no acid-fast bacilli. Culture of the sputum showed Streptococcus viridans and Staphylococcus aureus.

The patient made an immediate clinical response to sulfadiazine. Her temperature became normal on the third day. The sputum gradually decreased in amount and became free of blood; however, after a month she continued to raise a half-cup of mucoid sputum daily. The signs of consolidation in the upper lobe of the right lung persisted and were confirmed by roentgenogram. Two bronchoscopic examinations were done; nothing abnormal was found. Studies with iodized poppyseed oil 40 per cent were unsatisfactory. She received a course of treatment with penicillin, and postural drainage was instituted, without improvement. She was discharged to the chest clinic.

Permission to use the clinical records from the Chest Service of Bellevue Hospital was given by Dr. J. Burns Amberson.

The patient was hospitalized for the third time from Dec. 13 to Dec. 22, 1945. Since the previous discharge she had continued to have a cough productive of small amounts of mucoid sputum, and occasionally she suffered from dyspnea. One day before her admission fever, pleuritic pain and increasing dyspnea developed. There was no palpitation, nocturnal dyspnea, precordial pain or edema.

She was acutely ill, dyspneic and slightly cyanotic. Signs of consolidation were present over the entire right side of the chest. Both lungs were filled with bubbling rales and rhonchi. The heart seemed enlarged to percussion, with sounds obscured by the respiratory din. A roentgenogram of the chest revealed massive consolidation of the right lung, with deviation of the trachea to the right. The hemoglobin content was 11 Gm.; the white blood cell count, 6,000. The urine contained albumin (3 plus) with 25 red blood cells per high power field. The Wassermann test of the blood was negative. Culture of the sputum revealed Staph. aureus, Str. viridans and diphtheroids. The nonprotein nitrogen was 31 mg. per hundred cubic centimeters.

The patient was treated with a positive pressure oxygen mask, intravenous theophylline ethylenediamine injection, U.S.P., and penicillin and given rapid digitalization. In a day and a half the temperature fell to normal. However, the pulse rate remained about 120 per minute. Initially, the patient improved, and the lungs contained fewer rales. An inconstant expiratory wheeze was heard over the upper lobe of the right lung. The patient required continuous administration of oxygen, became worse, had increased rales in both lungs and died on the tenth day in the hospital.

The clinical diagnosis was lobar pneumonia, pulmonary fibrosis and emphysema, and bronchiectasis.

Necropsy.—The principal abnormal conditions were in the thoracic cavity. The visceral and parietal pleurae over the upper and middle lobes of the right lung were united by dense fibrous adhesions. The lower lobe of the right lung was covered by fibrinous exudate. There were no pleural adhesions over the left lung. Both pleural cavities were free of fluid. The trachea was deviated to the right.

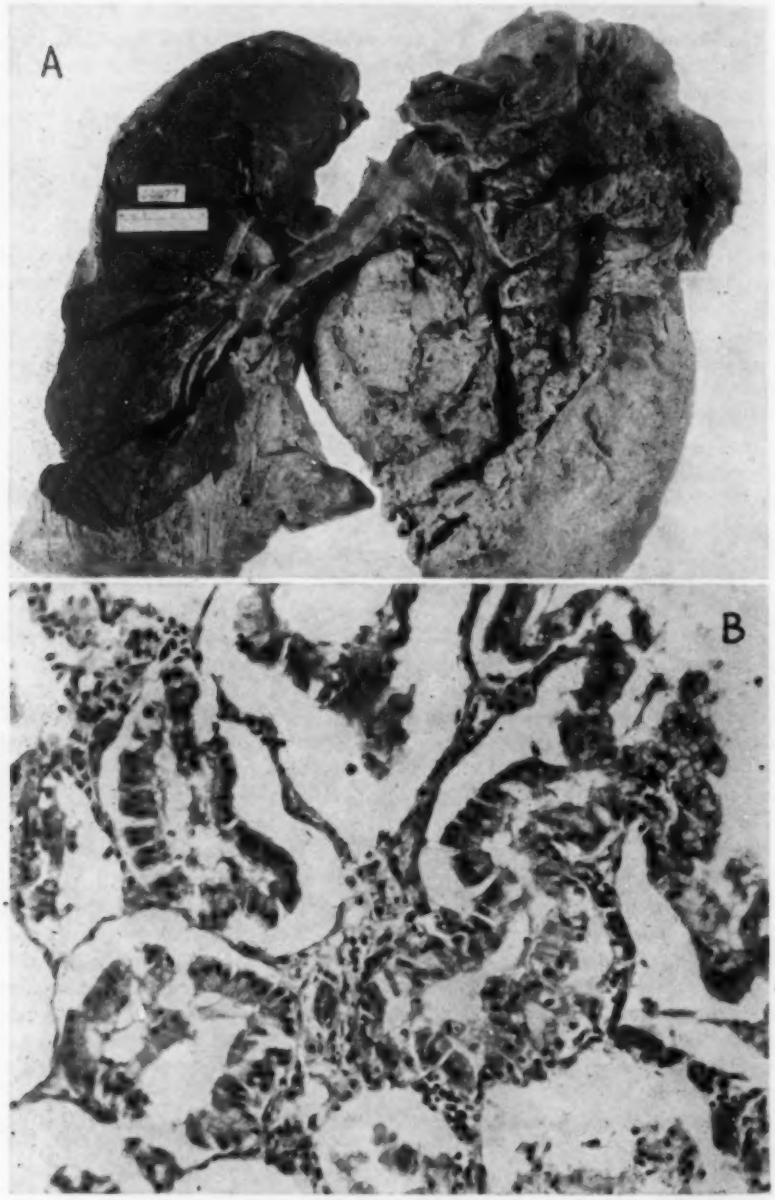

Fig. 1.—*A*, lobar consolidation and fibrinous pleurisy of the lower lobe of the right lung and bronchiectasis and fibrosis of the upper lobe of the right lung. *B*, lining of alveoli by nonciliated columnar epithelial cells forming mucin. From the lower lobe of the right lung; × 198.

The lungs together weighed 2,100 Gm., the right lung constituting about three quarters of the total weight. The upper and middle lobes of the right lung were small, practically airless and reddish gray on section. Their bronchi showed a fusiform dilatation.

The lower lobe of the right lung was large, heavy and consolidated throughout. The parenchyma was yellow-pink, with a moist, gelatinous cut surface. At the apex of the lobe there was necrosis of the parenchyma, with formation of a yellow-green exudate. The gross appearance of the lower lobe of the lung closely resembled the consolidation seen in pneumonia due to Friedländer's bacillus.

The left lung was of normal size, with a smooth gray pleura. On section the parenchyma was pink and crepitant. In the lower lobe of the left lung, about 2 cm. above the diaphragmatic pleura, there was a solitary nodule, 1 cm. in diameter, with a yellow gelatinous cut surface and a fairly firm consistence. This nodule had no demonstrable relationship to the bronchi or the bronchioles.

The bronchi of both lungs contained a small amount of viscous yellow fluid. At no point were the bronchi stenosed or ulcerated.

The peribronchial and tracheobronchial lymph nodes were small, soft and uniformly gray on section.

No similar tumor tissue was found in any other organ.

The spleen weighed 150 Gm.; microscopically, the red pulp was engorged with red blood cells, and there was an increased number of polymorphonuclear leukocytes. The liver showed a mild fatty change. A microscopic adenoma of islet tissue, about five times the size of a normal islet of Langerhans, was present in the pancreas. There was mild bilateral chronic salpingitis. No evidence of syphilis was found at necropsy.

Histologic examination of the lungs revealed a remarkable picture, varying according to the lobe sectioned.

In a section from the lower lobe of the right lung the pleura was engorged and covered by organizing fibrinous exudate. Nearly every alveolus contained fibrinopurulent exudate, and some of the alveolar septums were necrotic. The interlobular septums were edematous and contained neutrophilic leukocytes. Exudate containing epithelial cells was present in the bronchioles, the walls of which were acutely inflamed.

In many scattered foci, varying in diameter from 1 to 3 mm. in the stained preparation, the alveoli contained tall columnar cells that covered the alveolar septums in a single layer. The cells were larger than the columnar cells of the bronchial mucosa. Their nuclei were of moderate size, oval, basally situated in the cells, and had abundant chromatin, finely dispersed. Occasional nuclei had single basophilic nucleoli. The cytoplasm was abundant, homogeneous and eosinophilic. Most of these cells contained droplets of basophilic secretion in the cytoplasm near their free margins. Many alveoli lined by these cells were filled with basophilic secretion. The cells were nonciliated. Mitoses were not seen. There was considerable shrinking of these cells away from alveolar septums; this was considered to be largely an artefact. There was no papillary formation.

A mucicarmine stain demonstrated droplets of mucin within the cells and abundant mucin in the lumens of tumor-line alveoli.

A Wilder-Van Gieson stain for reticulum revealed a few reticulum fibers between the basilar portions of the lining cells in addition to the normal reticulum framework of the alveolar septums. Some of the tumor cells contained argyrophilic granules in the apical portions of the cells; these granules corresponded in location to the droplets of mucin seen with the mucicarmine stain.

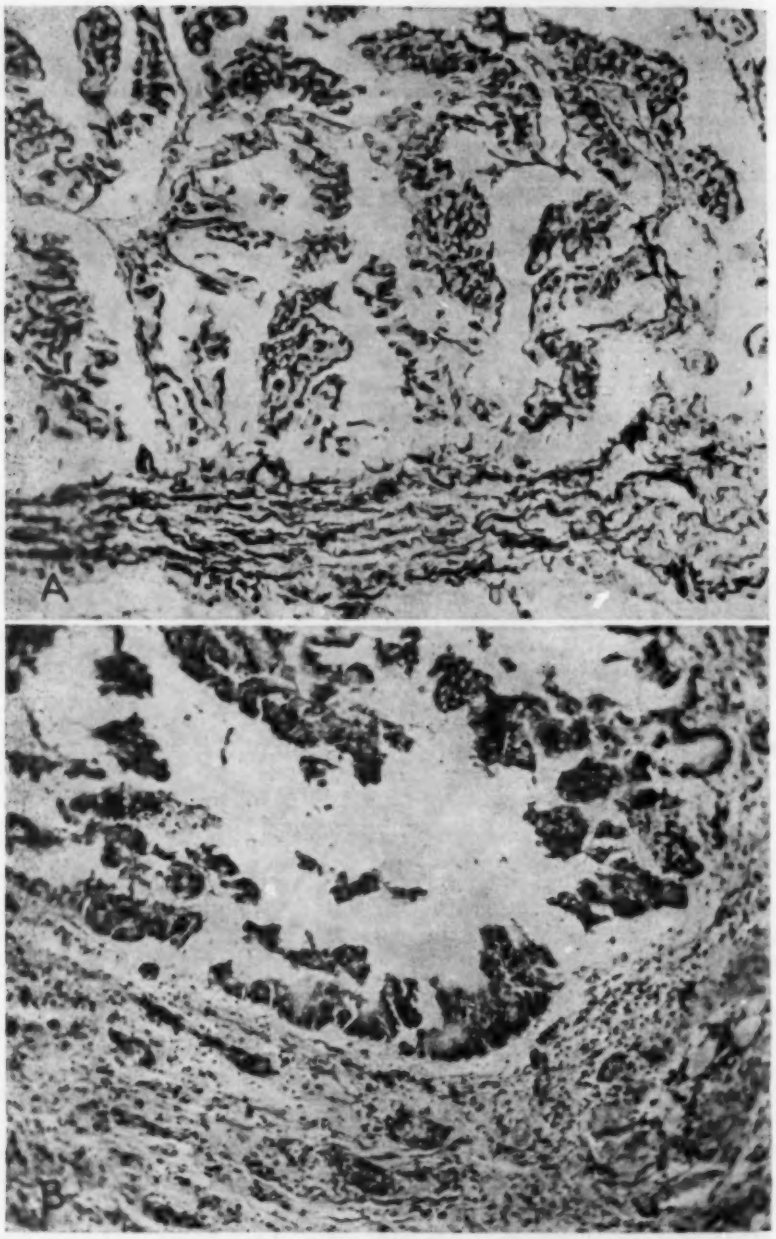

Fig. 2.—Wilder-Van Gieson stain for reticulum. No increase in fibrous tissue is present in the alveolar septums. The adjacent interlobular septum is not invaded. × 198.

B, space lined by mucin-secreting epithelial cells in the fibrotic upper lobe of the right lung. × 108.

A Brown-Breu stain revealed numerous and varied organisms in the pneumonic exudate, including gram-positive cocci (some in short chains), rare gram-positive diplococci and numerous short gram-negative bacilli.

Sections from the upper and middle lobes of the right lung revealed extensive fibrosis of the pleura and the parenchyma, with infiltrating neutrophils, lymphocytes and plasma cells. There were dilated bronchioles having a thick epithelial basement membrane and chronically inflamed walls. Also, large spaces were lined by columnar tumor cells and contained mucin and purulent exudate. These spaces lacked the muscle fibers and epithelial basement membrane of bronchioles.

Sections of the main bronchi in the upper and lower lobes of the right lung showed no neoplastic involvement.

A section from the upper lobe of the left lung was free of tumor.

A section of the nodule in the lower lobe of the left lung showed it to be composed of tumor-lined alveoli filled with mucin. The mucin had overflowed into alveoli that were free of tumor.

In none of the sections was it possible to demonstrate that the columnar cells lining alveoli were continuous with the bronchiolar epithelium. The epithelium lining the bronchi and the bronchioles was not hyperplastic. No invasion of blood or lymphatic vessels was seen.

Several sections of peribronchial and tracheobronchial lymph nodes showed no metastases; neither were metastases found in sections of other organs, nor in those of the vertebral marrow.

The anatomic diagnosis included: pulmonary adenomatosis of the upper, middle and lower lobes of the right and the lower lobe of the left lung; bronchiectasis and fibrosis of upper and middle lobes of the right lung; lobar pneumonia, lower lobe of the right lung; fibrinous pleurisy, lower lobe of the right lung; acute splenic tumor; adenoma of an islet of Langerhans; fatty liver, mild; chronic salpingitis.

COMMENT

The clinical course in this case was characterized by two episodes of pneumonia eleven months apart, the second episode ending in death. A roentgenogram taken following the first illness disclosed an unresolved process in the right upper lung field. At necropsy the upper and middle lobes of the right lung were fibrotic and contained tumor. We think it probable that the evolution of the tumor occurred during or even before the onset of the first episode of pneumonia. It seems unlikely that pneumonia contributed to the genesis of the tumor, since at necropsy there was a nodule of tumor in the lower lobe of the left lung, where there was no evidence of inflammation or fibrosis.

Indeed, it seems probable that the multiple tumors, with their abundant production of mucin, predisposed the involved parenchyma to infection. Some experimental evidence may support this hypothesis. Nungester and his co-workers [10] found that in rats an intrabronchial injection of mucin increased the incidence of pneumonia caused by pneumococci injected intrabronchially or intravenously.

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10. Nungester, W. J., and Jourdonais, L. F.: J. Infect. Dis. **59**:258, 1936. Kempf, A. H., and Nungester, W. J.: Proc. Soc. Exper. Biol. & Med. **43**:627, 1940.

The gross resemblance of the lower lobe of the right lung in our case to a lung with Friedländer's pneumonia duplicates a previous observation in 2 cases reported by Taft and Nickerson.[7]

There is no evidence that syphilis contributed to the genesis of this patient's pulmonary disease; a positive serologic test has not been reported in other published cases. The pancreatic islet tumor was apparently silent clinically.

Clinically, this disease simulates a chronic infectious disease of the lungs. Although clinical data are not available in all reported cases, it has been noted that cough was present in all cases in which mention was made of this symptom. Other symptoms usually present are dyspnea and loss of weight. Thoracic pain was described in 2 cases (Bonne[2]; Sims[5]). Hemoptysis was reported in only 1 case (Wood and Pierson[8]). The age of the patients has varied from 21 to 79 years; the sexes have been equally represented.

The average duration of the disease appears to be several months. Oberndorfer's[2] patient, a man aged 21, died after a brief illness, and, in addition to multiple foci of tumor, had extensive hemorrhagic pneumonia. On the other hand, Bell's[6] patient had symptoms of cough, dyspnea and loss of weight at least two and a half years before death. Most of the patients had pertinent symptoms at least nine months before death.

The clinical course had apparently never been sufficiently typical to make possible a correct diagnosis before pathologic study was possible.

The prognosis of this disease appears to be poor, the patient dying either of superimposed pneumonia or of respiratory insufficiency resulting from the lining of alveoli by tumor cells. In the only patient treated surgically by lobectomy, other lobes were subsequently found to be involved by the tumor.[8]

In the literature a lively discussion had occurred on three points: (1) the origin of the tumor cells; (2) the nature of the tumor, benign or cancerous; (3) the possible relationship of the tumor to an anatomically similar infectious disease of sheep sometimes called "jagziekte."

Origin of the Tumor Cells.—Do they arise in the alveoli or are they derivatives of bronchiolar epithelium? This point is unsettled, since opposing observers are not in agreement even as to the validity of some of the evidence presented. The subject is well reviewed by Geever, Neubuerger and Davis.[11]

Even the debate whether alveolar lining cells occur in the adult human lung is unsettled. Bell[6] and Geever, Neubuerger and Davis[11] studied the lung as affected by chronic passive congestion and inflammatory condi-

11. Geever, E. F.; Neubuerger, K. T., and Davis, C. L.: Am. J. Path. **19**:913, 1943.

tions. They observed epithelial proliferation from alveolar lining cells. They concluded that epithelial cells occur in the alveoli of the normal adult human lung but do not form a continuous lining. In their opinion these cells may give rise to tumors. Recently, however, Herbut [12] studied regenerating epithelium lining alveoli in cases of bronchiectasis by serial sections and concluded that the regenerated epithelium was derived from bronchiolar epithelium.

The distribution of the tumor in multiple foci, lining alveoli and without demonstrable continuity with bronchiolar epithelium, argues for an alveolar origin of the growth. This view has been taken by, among others, Bell,[6] Geever, Neubuerger and Davis,[11] Taft and Nickerson [7] and Wood and Pierson.[8]

It appears that in animals similar tumors arise from alveolar cells. Cowdry and Marsh [13] have studied jagziekte in sheep and concluded that the proliferated cells arise through "a metamorphosis of alveolar epithelium." Grady and Stewart [14] produced multiple tumors in the lungs of mice by injecting subcutaneously 1,2,5,6-dibenzanthracene and methylcholanthrene. In sections taken at different intervals following injection, they traced the evolution of epithelial tumors from proliferated cells in the alveolar septums.

The columnar type of tumor cells in human pulmonary adenomatosis, with abundant production of mucin, led Richardson [4] to favor an origin from bronchiolar epithelium.

Recently, Herbut has denied the existence of tumors derived from alveolar cells. The capacity to line alveoli does not prove the alveolar origin of the cells, he observed, because such lining tendency is seen occasionally in carcinoma proved to be bronchogenic and in adenocarcinoma of the lung secondary to carcinoma of the gallbladder, the colon and the pancreas. He pointed out that overgrowth of the tumor may obscure the point at which it originates from bronchial or bronchiolar epithelium. He also stated that the fact that there are multiple foci in the lungs does not prove a multicentric origin of the tumor, since the tumor may spread within the lung via lymphatics or via bronchial or bronchiolar lumens.

We believe that in our case the tumor was most likely of multicentric origin since there was no demonstrable invasion of lymphatic vessels. The idea that the tumor spreads by bronchiolar lumens is purely hypothetic.

Evidence for Classification of the Tumor as Benign or Cancerous.—In the 9 cases cited by us from the literature the growth was adenoma in the sense that it was composed of columnar cells lining alveoli and

12. Herbut, P. A.: Am. J. Path. **20**:911, 1944; Arch. Path. **41**:175, 1946.
13. Cowdry, E. V., and Marsh, H.: J. Exper. Med. **45**:571, 1927.
14. Grady, H. G., and Stewart, H. L.: Am. J. Path. **16**:417, 1940.

having usually the capacity to secrete mucin. There were no metastases even in the regional lymph nodes, whereas bronchogenic carcinoma is known to involve lymph nodes early.

In most of the reported cases the cells have been rather uniform, with rare or no mitoses.

Local invasiveness was observed only in the case reported by Bonne, in which the pleura was involved. Vascular invasion has been absent.

All of these features, in our opinion, favor the view that this is a benign tumor of multicentric origin, aptly termed "pulmonary adenomatosis."

It is true that similar cases have been reported in which metastases were found in lymph nodes or distant organs (Weissmann [15a]; Briese [15b]; other cases cited by Neubuerger and Geever). A review of the histologic descriptions and illustrations of these cases leads us to conclude that in most instances of "alveolar cell" carcinoma the neoplasm is more plemorphic, with numerous mitoses, nuclei larger in proportion to the cells and often bizarre giant nuclei. However, the resemblance between pulmonary adenomatosis and metastasizing alveolar cell tumor is sufficiently close to suggest the possibility that pulmonary adenomatosis is a potentially malignant tumor.

Resemblance to Jagziekte.—Jagziekte is an infectious disease of sheep, of unknown etiologic agent, occurring in Iceland, South Africa and England. A similar infectious disease, "progressive pneumonia," occurs in sheep in Montana. Cowdry and Marsh [13] have studied these conditions extensively. The disease lasts several months and is always fatal, death occurring apparently from asphyxia. At necropsy the lungs disclose "chronic catarrhal pneumonia," with many alveoli lined by cuboidal and columnar cells, which often show papillary infolding into alveolar lumens. Mucin formation is not prominent. The tumor is not invasive; in only 1 case, reported by Aynaud (cited by Dungal,[16]) have metastases been found in regional lymph nodes. The similarity of the disease to human pulmonary adenomatosis has been mentioned repeatedly. For a fuller discussion, the reader is referred to the articles of Bonne [3] and Dungal.[16]

No person with pulmonary adenomatosis has ever been proved to have been exposed to diseased sheep, and most cases of human adenomatosis have been reported in localities where the ovine disease is unknown.

Thus, while a symptomatic and anatomic similarity exists between human and ovine adenomatosis, there is no evidence that the two diseases have a common cause.

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15. (*a*) Weissmann, S.: Frankfurt. Ztschr. f. Path. **47**:534, 1935. (*b*) Briese: Frankfurt. Ztschr. f. Path. **23**:48, 1920.

16. Dungal, N.: Proc. Roy. Soc. Med. **31**:497, 1938.

SUMMARY

Pulmonary adenomatosis involving four lobes of the lungs has been observed.

It is probable that the multiple tumors, producing abundant mucin, predisposed the lungs to pneumonic infection.

It remains uncertain whether in pulmonary adenomatosis the tumor cells arise from alveolar lining cells or from bronchiolar epithelium.

This disease resembles infectious adenomatosis of sheep, although proof is entirely lacking that the two diseases are related etiologically.

SYSTEMIC INFECTION WITH CRYPTOCOCCUS NEOFORMANS (TORULA HISTOLYTICA) AND HISTOPLASMA CAPSULATUM IN THE SAME PATIENT

G. B. MIDER, M.D.; F. D. SMITH, M.D., and W. E. BRAY Jr., M.D.
CHARLOTTESVILLE, VA.

Mixed infection with pathogenic fungi is of extreme rarity.

REPORT OF A CASE

B. H., a 12 year old Negro boy, was admitted to the medical service of the University of Virginia Hospital, complaining of weakness and loss of weight of two or three months' duration. The history obtained was meager because of the patient's debility.

He apparently enjoyed good health until approximately three months before admission, when there was an insidious onset of weakness, anorexia, progressive loss of weight and night sweats. Five to six weeks before admission a red, painful swelling developed over the right sternoclavicular joint, which was incised by his local physician one month before admission. A yellow mucoid material was obtained, which was said to be sterile. The incision failed to heal, and the lesion continued to drain. Two weeks before admission the patient noted a nonproductive cough and began to bleed from the gums. All of his symptoms progressed, and during the week prior to admission he became so weak that he could not walk.

When examined by us the patient was extremely emaciated and acutely ill. His temperature was 100.2 F. (38 C.), pulse rate 120, respirations 32 and blood pressure 95 systolic and 40 diastolic. The skin was dry and scaly. The mucous membranes were pale. Several teeth were crusted with blood. There was general lymphadenopathy; the involved nodes were nontender. Over the right clavicle just lateral to the sternum there was an indurated, tender area 4 by 5 cm., in the center of which was an ulcer about 1 cm. in diameter. Thick sanguinous fluid drained from the ulcer. The spleen extended 3 cm. below the costal margin. The liver was firm and extended 8 cm. below the right costal margin. There was no evidence of ascites. Slight edema was present over the shins. The heart and the lungs were essentially normal.

The hemoglobin content (Haden-Hauser method) was 5.25 Gm.; the erythrocyte count, 1,940,000; the leukocyte count, 2,400, with segmented neutrophils 46, band cells 44, lymphocytes 2 and monocytes 8 per cent. The neutrophils showed moderate toxic granulation. One normoblast was seen per hundred leukocytes. The blood platelets appeared to be moderately decreased in number. The bleeding time (Duke method) was five and a half minutes; the clotting time (capillary tube method), five minutes. The reticulocyte count was 5 per cent. The sickle cell trait was absent. There was a slight trace of albumin in the urine, and the urinary sediment contained occasional leukocytes, erythrocytes and finely granular casts. The benzidine test of the stool was weakly positive for occult blood. No ova or parasites were found. The Wassermann test of the blood was negative.

From the Departments of Pathology, Clinical Pathology and Medicine, University of Virginia School of Medicine.

The roentgenologist who examined the chest reported as follows: "The lung fields, especially the left, have hazy spotty densities, with very rounded infiltrations around the lung markings, about 1 to 2 mm. in diameter." This was interpreted as possible leukemic infiltration of the left lung.

Leukocyte concentration smears were made because of the marked leukopenia and anemia associated with hepatic and splenic enlargement. They revealed the organisms of Histoplasma capsulatum in occasional monocytes (fig. 1). Similar organisms were found without difficulty in smears made from the sternal marrow (fig. 2). These organisms were found in the peripheral blood smears made the night of admission but were not recognized at that time.

The patient's course in the hospital was progressively down hill. Anorexia was marked. Treatment consisted entirely of supportive measures. He received three blood transfusions, ferrous sulfate and supplementary vitamins, including vitamin K. During his stay in the hospital his temperature varied between 99 and 102 F. (37.2 to 39 C.). His pulse rate averaged 130 and the respiratory rate 36. The lesion over the right clavicle continued to bleed. Topical applications of a thromboplastic substance controlled the bleeding temporarily. Decubital ulcers appeared over the pressure points on his back. He died a few hours after the onset of delirium eleven days after admission to the hospital.

Necropsy (thirty-six hours after death).—The fat depots throughout the body were depleted. An ovoid granular red ulcer, measuring 10 by 14 mm., overlay the eroded surfaces of the right sternoclavicular joint. Three superficial decubital ulcers were seen on the back.

The skeletal muscles were well formed, pink and flabby. The axillary and inguinal lymph nodes were dark red and soft, with well defined cortical and medullary zones. They averaged 1 to 1.5 cm. in the greatest dimension.

There were 500 cc. of clear, bright yellow, watery fluid in the peritoneal cavity, 60 cc. of similar fluid in each of the pleural cavities and 100 cc. in the pericardial sac. The linings of these spaces were smooth, translucent and glistening.

The pale, flabby but well formed heart weighed 135 Gm. It contained soft sedimented clots.

The soft bilobed thymus which weighed 5 Gm. had a finely granular, pink cut surface.

The voluminous lungs together weighed 860 Gm. The pleurae contained numerous punctate hemorrhages. The anterior third of the pulmonary tissue was pink, reticulated and crepitant. The remainder of the lung was dusky purple, boggy and nonaerated. Copious, bright red, frothy fluid exuded from the cut surfaces, which were gray-red, spongy and marked with many slightly raised darker irregular areas 3 to 7 mm. in diameter. These lesions, more numerous near the hilus, were poorly delimited and seemed to be peribronchial in distribution. Some of them had tiny central yellow areas. The trachea and the bronchi contained frothy fluid. Their mucosae were yellow-red. The tracheobronchial lymph nodes varied from 2 to 4.5 cm. in the greatest dimension. They had red-gray, finely granular cut surfaces.

The large, pale liver weighed 760 Gm. A few gray subcapsular opaque areas, 2 mm. in diameter, were seen in the right lobe. Yellow material present in the periphery of each lobule accentuated the normal markings throughout the organ. A solitary, soft, bright yellow, structureless area 4 mm. wide surrounded by a thin fibrous capsule lay in the right lobe. The extrahepatic biliary system was normal.

The enormous, purple, firm spleen, which weighed 760 Gm., had a dark red homogeneous cut surface from which the pulp could be scraped easily.

No changes were recognized in the pancreas.

The esophagus, the stomach, the duodenum and the proximal half of the jejunum did not appear remarkable. In the distal half of the jejunum and in all of the ileum, on the free edges of the semicircular plicae were discrete firm gray nodules from 2 to 3 mm. in diameter. Similar nodules studded the mucosa of the colon. They were most numerous in the ileum, the cecum and the ascending colon and became gradually less plentiful as one proceeded in either direction from these regions. Some of the masses were surrounded by hyperemic halos, some appeared to be superficially ulcerated, and on cut section all were gray and structureless, although a few of the largest had small yellow cores. These lesions did not correspond to the distribution of intestinal lymphoid follicles, which were readily recognized.

The largest lymph nodes in the body lay in the mesentery and about the head of the pancreas. Many of them attained a diameter of 4 cm. and were gray-red and granular. The largest nodes had yellow, dry, structureless centers suggestive of caseous necrosis.

The adrenal bodies, which together weighed 15 Gm., were yellow-gray, marked with several bright yellow, sharply circumscribed, structureless lesions 1 to 2 mm. in diameter, confined to the cortical tissue.

Each of the pale kidneys weighed 90 Gm. Their capsules stripped readily, leaving smooth yellow-red cortical surfaces mottled with punctate hemorrhages near the lower poles. Similar hemorrhages were seen on the cut surfaces, in which cortical and medullary zones were well defined but the columns of Bertini were obscured. A dry yellow area of necrosis replaced the right lower renal papilla. The renal pelves and ureters appeared normal. The mucosal surface of the urinary bladder was studded with minute hemorrhages.

The external and internal genitalia, prepubertal in type, showed no changes of pathologic significance.

The leptomeninges were opaque and slightly thickened over the convexity of the cerebrum. Multiple sections of the well formed brain failed to reveal any structural changes. The pituitary body appeared normal.

A soft yellow area 1.7 cm. in diameter, with depressed center and scalloped edges, lay in the left frontal bone, extending from the galea aponeurotica to the dura mater. A similar area 8 mm. in diameter was found in the body of the tenth thoracic vertebra. It was surrounded by a thin zone of pale gray tissue. Elsewhere the vertebral marrow was red-gray and the femoral marrow, red-brown and gelatinous.

Microscopic Examination.—Organisms of two morphologically separate and distinct types, belonging to the fungi imperfecti, were found in the tissues of this patient. Those of the first type, which were more numerous, were basophilic, gram-positive spherical bodies 1 to 5 microns in diameter surrounded by thin achromatic halos. The central chromatin contained a small vacuole. No evidence of budding was found. These organisms were invariably intracellular but excited little inflammatory reaction. They conformed in their structural details to H. capsulatum.

The second type was doubly contoured, round or elliptic, gram-positive, from 5 to 25 microns in diameter, with little chromatin, and was surrounded by a thick, highly refractile capsule. Budding forms were present. These organisms were extracellular except for those that lay within foreign body giant cells. A peculiar

mucoid substance was present when the yeast cells were found in large numbers. The organisms were most numerous in the leptomeninges. They conformed in their structural detail to Cryptococcus neoformans (Torula histolytica).

In the heart no changes of pathologic import were found.

In the lungs, many of the small pulmonary veins contained small mural thrombi, and some were completely occluded. Macrophages filled with histoplasmas were conspicuous in the thrombi and in the necrotic walls of the affected vessels (fig. 3). Lymphocytes formed a cuff about the necrotic areas, and there were small hemorrhages in the surrounding tissue. In some sections the venous lesions were so numerous that the hemorrhages coalesced. Tiny foci of pneumonia with fibrin and a few polymorphonuclear leukocytes were seen near some of the hemorrhages, but neither pneumonia nor parenchymal necrosis was a conspicuous feature of the pulmonary change. Relatively uninvolved portions of the lung were edematous. No histoplasmas were seen in the bronchial epithelium and only a few were found in the cells lining the pulmonary alveoli. No cryptococci were found.

In the liver, the cytoplasm of practically every Kupffer cell was filled with histoplasmas (fig. 4). Smaller numbers of the organisms were seen in hepatic cells, but none were seen in the biliary epithelium. The isolated focus was a mass of coagulation necrosis and cryptococci surrounded by foreign body giant cells and enclosed in a thin capsule of dense collagenous tissue (fig. 5). Only a few histoplasma-laden macrophages were seen in this lesion. The hepatic structure was well retained. Parenchymal cells in the periphery of each lobule contained large intracytoplasmic sudanophilic vacuoles. The central zones were hyperemic, and some of the cells showed the early changes of necrosis.

The pulp of the spleen was intensely congested. Many of the splenic follicles were made up of reticulum cells with few lymphocytes. Large numbers of histoplasmas were found in the reticulum cells in the follicles and in those lining the sinusoids. No cryptococci were found.

Occasional foci of coagulation necrosis were found in parenchymal and interstitial tissue of the pancreas. The number of islets was reduced, and probably some of the necrotic foci represented damaged islets. Histoplasmas were seen within macrophages in the necrotic foci and in thrombosed veins. No cryptococci were found.

No pathologic changes were encountered in the stomach or the duodenum. There was advanced autolysis of the luminal cells throughout the intestines. Histoplasma-laden macrophages formed nodules confined to the tunica propria of the small bowel, particularly at the tips of villi. Some of the nodules had small central necrotic areas. Acute inflammatory reaction was absent. The lymphoid follicles were not involved. Similar lesions were seen in the mucosa and the submucosa of the colon.

In the adrenal glands cortical lipid was depleted. Two types of lesions were seen in the cortices. The smaller, generally 50 to 100 microns in diameter, were composed of groups of necrotic cells, histoplasma-laden macrophages and polymorphonuclear leukocytes. The larger lesions, visible macroscopically, were sharply outlined groups of swollen cells, probably macrophages, filled with histoplasmas. A few histoplasmas were seen also in adrenal cortical cells that appeared structurally normal. Small veins in the cortex and the medulla contained thrombi in which histoplasmas were found. No cryptococci were seen.

In the kidneys, small focal necroses containing histoplasma-laden macrophages distorted the cortical structure. Foreign body giant cells were seen about some of these foci. The lesions appeared to be vascular in origin, since renal venules were

thrombosed and one area of necrosis had the configuration of a small infarct. An encapsulated focus of cryptococci in the lower papilla of the right kidney resembled the lesion found in the liver. Histoplasmas were seen in structurally normal glomeruli, in epithelium lining proximal convoluted tubules and in normal endothelium lining veins in the absence of thrombosis.

The submucosa of the bladder contained focal accumulations of histoplasma-laden macrophages.

No pathologic changes were seen in the prostate.

In the testes, there were focal collections of histoplasma-laden macrophages in the tunica albuginea.

In the lymph nodes, the earliest change, seen best in the axillary and tracheobronchial lymph nodes, was a marked hyperplasia of sinusoidal endothelial cells, which were filled with histoplasmas. Lymphoid follicles were scarce and germinal centers absent. Small foci of necrosis were seen among the parasitized cells. The intra-abdominal nodes contained numerous focal necroses and large caseous areas. All of the organisms seen were typical histoplasmas except those in one node, where a large encapsulated area of cryptococci and foreign body giant cells embedded in a mucinous matrix was found.

No organisms were seen in the involuted thymus.

The leptomeninges were edematous, containing macrophages, few lymphocytes, polymorphonuclear leukocytes and focal areas of foreign body giant cells within which typical cryptococci were seen. Extracellular cryptococci lay in the meninges and filled some of the Virchow-Robin spaces (fig. 6). There were no parenchymal lesions. Histoplasma could not be identified with certainty in either the leptomeninges or the brain tissue.

The femoral marrow contained few granulopoietic cells, rare adult granulocytes and little fat. Normoblasts and earlier erythropoietic forms were numerous. The predominating cells were large and ovoid, with eccentrically placed nuclei and pale acidophilic cytoplasm filled with histoplasmas. They were thought to be a mixture of reticulum cells and macrophages. Only scattered small foci of hemopoiesis were present in the vertebral marrow, which was largely replaced by histoplasma-laden cells like those in the femoral marrow. The region of the abscess in the tenth thoracic vertebra contained large numbers of cryptococci mixed with

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EXPLANATION OF FIGURES 1 TO 12

Fig. 1.—Yeast form of H. capsulatum in the cytoplasm of a monocyte in a concentration smear of peripheral blood; Wright's stain; × 1,055.

Fig. 2.—Yeast form of H. capsulatum in the cytoplasm of a monocyte from a smear of sternal marrow; Wright's stain; × 1,055.

Fig. 3.—Small pulmonary blood vessel showing a mural thrombus containing histoplasmas; hematoxylin and eosin; × 234.5.

Fig. 4.—Histoplasmas in Kupffer cells of the liver; hematoxylin and eosin; × 234.5.

Fig. 5.—Cryptococci in a necrotic focus of the liver; hematoxylin and eosin; × 234.5.

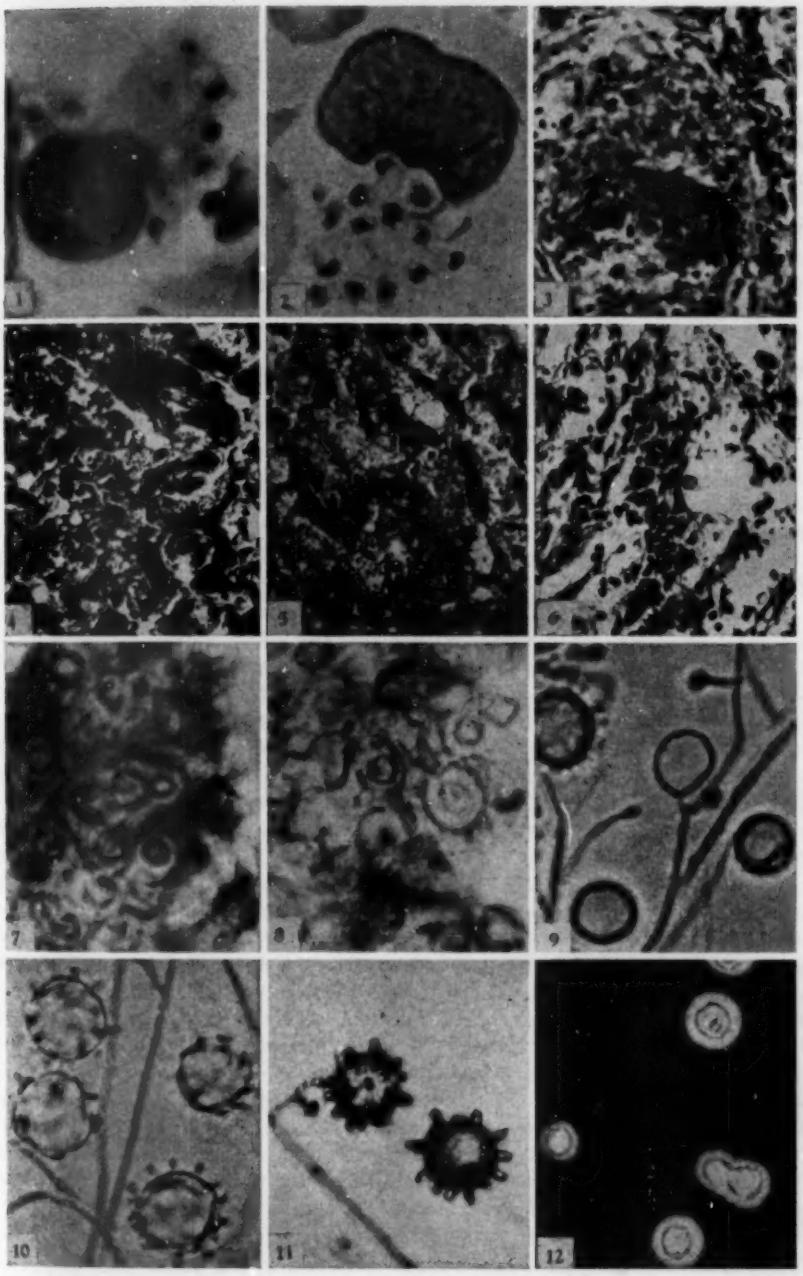
Fig. 6.—Cryptococci in the leptomeninges; hematoxylin and eosin; × 234.5.

Figs. 7 and 8.—Cryptococci in pus, showing capsules; Greenthal's stain of a wet preparation; × 539.

Figs. 9 and 10.—H. capsulatum from a slide culture, showing different stages in the development of tuberculate chlamydospores; × 539.

Fig. 11.—H. capsulatum from a slide culture, showing characteristic brown tuberculate chlamydospores; × 539.

Fig. 12.—C. neoformans from a three day old growth on Sabouraud's agar; india ink preparation showing capsules; × 539.



(See legends on opposite page)

a few histoplasmas. Many of the bony trabeculae had a mosaic pattern due to the presence of hyaline cartilage. The right sternoclavicular joint space was filled with cryptococci and a few intracellular histoplasmas. Two thirds of the articular surface of the clavicle was eroded. Marrow spaces in the epiphysial remnant were fibrosed. Short, blunt mosaics of bone and cartilage lay in the region of endochondral ossification. Histoplasmas and cryptococci were found throughout the clavicular marrow, but the latter were particularly numerous in the metaphysial portion.

Mycologic Studies.—With a pipet, material was aspirated from the draining lesion in the region of the right clavicle. This serosanguinous exudate contained clumps of pus cells among which were scattered single and budding yeast cells. Greenthal-stained wet preparations showed that the largest yeast cells exceeded the leukocytes in size and had sufficiently wide capsules to suggest C. neoformans (T. histolytica) (figs. 7 and 8).

Wright-stained smears of the pus showed many single and budding yeast cells which varied greatly in size. The smaller ones were frequently found within the cytoplasm of monocytes and occasionally in neutrophils. They stained blue and were usually 2 to 4 microns in diameter, but occasionally their size equaled that of the red blood cell. Except for the marked variation in size, the intracellular organisms closely resembled the histoplasmas seen in the blood smears. It was noted, however, that some of the nuclei were round with achromatic centers instead of having the usual crescentic shape. The extracellular organisms varied in size from those previously described up to large single budding forms 10 to 12 microns wide and 15 to 18 microns long, including the capsules. The width of the pink-stained capsule was about one-fourth the diameter of the blue-stained yeast cell but became progressively less distinct as the organisms became smaller. In the larger yeast forms the pink area surrounding the cell quickly faded peripherally into a relatively broad achromatic zone, the outer border of which was outlined by the surrounding cells or sometimes by a line of finely precipitated stain.

In culturing the blood the centrifuged sediment of oxalated blood remaining after a portion of the leukocyte-containing layer had been removed for concentration smears was poured into two blood agar plates which were sealed and then incubated at 37 C. A week later several minute yeastlike colonies which had appeared were transplanted to Sabouraud's agar test tube slants, and the plates were left at room temperature. Fuzzy white mycelial colonies were first seen on one of the blood agar plates ten days after inoculation. They were transplanted to three van Tieghem's cultural cell slides prepared with Sabouraud's agar. The first culture to show tuberculate chlamydospores characteristic of H. capsulatum was a yeastlike transplant on Sabouraud's agar after one month's growth (figs. 9 and 10). A van Tieghem's cell transplant of this culture made four days before the development of definite tuberculations on the chlamydospores, however, was characteristic of H. capsulatum within nine days. The variability in the time required for the development of tuberculate chlamydospores is further illustrated by the contrasting observation that two slide cultures made by direct inoculation from three week old blood agar plate colonies required six weeks for maturation. The tuberculate chlamydospores became brown in about one month (fig. 11).

Organisms of one of the yeastlike colonies early transplanted from the blood agar remained in the yeast form at room temperature both in old (two and one-half month) cultures and in a series of frequently transplanted colonies. India ink preparations were then made of one of the latter, and among many single-budding yeast cells were seen a few large encapsulated forms typical of C. neoformans.

Exudate from the draining area in the right clavicular region was inoculated on blood agar, and yeastlike organisms were isolated from this culture. Numerous transplants were made, including slide cultures. With two exceptions the organisms remained in the yeast form at room temperature, both in old (five month) and in frequently transplanted cultures. The yeast was equally luxuriant in the incubator (37 C.) and at room temperature. The growth initially was white or cream colored and turned to light brown in a few months. India ink preparations of a five day old, fourth generation blood agar transplant showed that the larger yeastlike cells had well developed capsules characteristic of C. neoformans (fig. 12). The capsules of these organisms are frequently smaller when examined in cultures than when examined in pus or spinal fluid.

Two van Tieghem's cell Sabouraud agar slides, A and B, were inoculated from an original blood agar culture. Mycelial growth appeared after one week in A. Many terminal chlamydospores were present within two weeks. These, however, did not show definite tuberculations characteristic of Histoplasma until two months later. Two transplants made in the interim from this slide culture grew out as encapsulated yeast cells. Hence, it is inferred that this was a mixed culture of Histoplasma and Cryptococcus. (See animal inoculations.) Slide culture B showed mycelial growth with the development of smooth-walled chlamydospores which never developed to the tuberculate stage.

Examination of a two month old slide culture transplant from A showed that some of the larger thick-walled yeast cells had given rise to rudimentary coarse filaments. Similar observations have been made on Cryptococcus cultures by Weidman[1] and Conant.[2]

Animal Inoculations.—The first inoculations carried out were with the yeast form of the fungus obtained by culture of pus. One cubic centimeter of a saline suspension made from the surface growth on a fourth generation Sabouraud's agar slant was injected intraperitoneally into each of 2 mice. One of the mice died twelve days after inoculation, and the other was obviously ill when killed and examined two days later. Cultures were made of material from the spleen and the liver on Sabouraud's agar. A luxuriant yeast growth was obtained in each case, and india ink preparations of the splenic culture contained, among many smaller single-budding yeast cells, a few large forms with well developed capsules characteristic of C. neoformans (T. histolytica).

Other inoculations of mice were carried out as follows: 1. The inoculum consisted of yeast recovered from a Sabouraud's agar transplant from slide culture A. The mouse was moribund on the seventeenth day, when it was killed and an autopsy made. Blood agar cultures of spleen showed single-budding yeast cells four days later. Several of the cells had large capsules. 2. The inoculum was a Sabouraud's agar culture of yeast isolated from the patient's blood. This mouse died seventeen days after inoculation, and cultures were made at autopsy. India ink preparations again indicated C. neoformans, and a majority of the yeast cells showed well developed capsules in a culture five months old. 3. A saline suspension prepared from a fifth generation Sabouraud's agar transplant of H. capsulatum from a blood culture was used as the inoculum. The mouse never showed any signs of

1. Weidman, F. D.: Pathogenic Yeasts, Molds and Actinomycetes, in Park, W. H., and Williams, A. W.: Pathogenic Micro-Organisms, ed. 11, Philadelphia, Lea & Febiger, 1939.

2. Conant, N. F., and others: Manual of Clinical Mycology, Philadelphia, W. B. Saunders Company, 1944.

illness, and at autopsy, six weeks later, no macroscopic or microscopic lesions were found. Cultures of the omentum, however, were identical with previous cultures of Histoplasma.

The histologic changes observed in the mice that were inoculated intraperitoneally with cultures resembling C. neoformans were similar. Brain, liver, spleen, lungs, kidneys, omentum, intra-abdominal lymph nodes and retroperitoneal fat contained cystlike spaces as much as 3 mm. wide filled with encapsulated yeast cells varying from 3 to 25 microns in diameter. The yeast cells had round nuclei. They were always extracellular, and despite the large size that some of the lesions attained, inflammatory reaction was minimal. Numerous organisms were found in the leptomeninges. The distribution and the appearance of the lesions conform to those described by Kessel and Holtzwart [3] as characteristic of the reaction to Cryptococcus. The results of our inoculations of H. capsulatum, as already indicated, agree with those of Parsons,[4] who has shown that intraperitoneal injection of H. capsulatum produces no lesions in mice. Intravenous inoculations produce systemic disease, but these were not attempted.

COMMENT

The differences in the structure and the cultural characteristics of the two yeasts isolated from this patient and in the lesions produced both in the patient and in mice by them indicate clearly that a mixed infection existed. Apparently the intestinal tract served as the portal of entry for the Histoplasma, since the oldest lesions were found in the ileum and the mesenteric lymph nodes. When parasitized macrophages were disseminated by way of blood and lymph channels, widespread changes were produced, among which venous thrombosis was prominent. It is probable that the mechanism by which the thrombi were formed involves the engulfing of histoplasmas by endothelial cells with subsequent damage to the vascular lining and thrombus formation. The portal of entry for the crytococci seems less clear, although a single intra-abdominal lymph node contained a large focus of these organisms. It may be inferred that the patient was infected simultaneously by both H. capsulatum and C. neoformans. The recognized predilection of cryptococci for the leptomeninges was well illustrated. Only a few small foci of crytococci were found elsewhere, and most of these also contained histoplasmas.

Little is known of the natural reservoirs of either Cryptococcus or Histoplasma, but concomitant infection with two different fungi, a rarity even among dermatomycoses,[5] suggests that they may have come from a common source.

SUMMARY

A Negro boy died approximately three months after the onset of a subacute febrile illness. C. neoformans (T. histolytica) and H. capsulatum were grown from the blood and from exudate. Lesions produced by each of these fungi were found at autopsy.

3. Kessel, J. F., and Holtzwart, F.: Am. J. Trop. Med. **15**:467, 1935.
4. Parsons, R. J.: Arch. Path. **34**:229, 1942.
5. Muskatblit, E.: Arch. Dermat. & Syph. **44**:631, 1941.

MYELOFIBROTIC ANEMIA IN HODGKIN'S DISEASE

ELWYN L. HELLER, M.D., AND GILMORE M. SANES, M.D., PITTSBURGH

ANEMIA is so constant a finding in Hodgkin's disease, particularly in the terminal stages, that it can be considered an integral feature of the disease. In the usual case it is of "secondary" type, not particularly severe, and is vaguely attributed to nutritional, toxic or infectious factors or, less specifically, it is considered to be an inevitable accompaniment of the cachexia which characterizes the disease. Although involvement of the skeletal system is a common complication of Hodgkin's disease, it frequently represents an incidental postmortem observation, its incidence increasing in direct proportion to the diligence of the examination. Less frequently, involvement of bone is an important feature of the clinical picture, producing pain, with destructive infiltration readily demonstrable by roentgenologic examination. It appears that severe anemia of myelofibrotic [1] type resulting from widespread involvement of the hemopoietic marrow is unusual in Hodgkin's disease.

REPORT OF A CASE

Miss I. H., a 60 year old woman, was admitted to the service of Dr. C. W. Morton at the Presbyterian Hospital on April 23, 1945 with a history of fever for a period of one month preceding admission. Her temperature rose to 100 or 101 F. daily. The remainder of the recorded history was irrelevant except for nocturia of many years' duration and occasional hematuria.

The patient was well developed and well nourished. No physical abnormalities were observed. The red blood cells numbered 3,500,000 per cubic millimeter; the hemoglobin content was 70 per cent; the white blood cells numbered 5,000 per cubic millimeter. The differential count was normal. The sedimentation rate was 18 mm. in thirteen minutes.

Throughout the one week period of hospitalization there was a low grade fever, with a maximum temperature of 101 F.

On July 9 the patient was readmitted complaining of fever and chills of two days' duration. Since leaving the hospital she had noticed occasional ecchymotic areas in the skin.

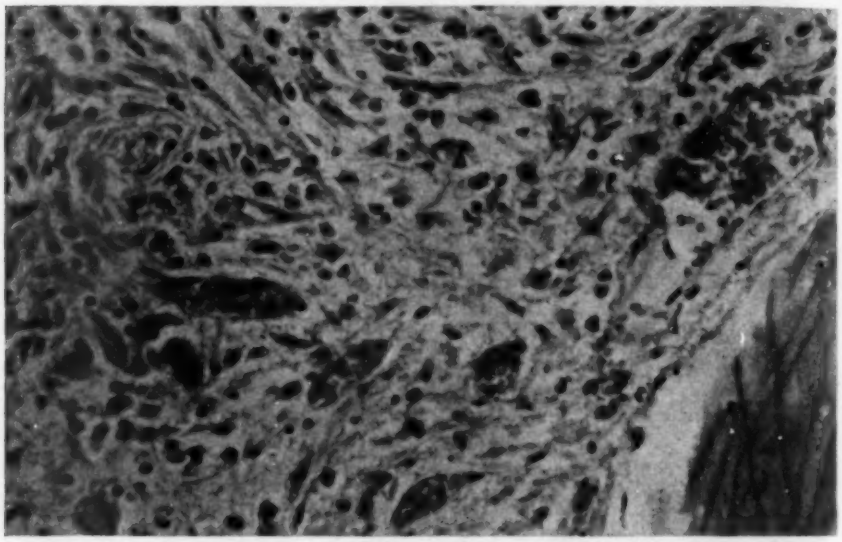
The physical findings were normal except for scattered ecchymoses. The red blood cell count varied between 3,500,000 and 4,000,000; the hemoglobin content was 70 to 80 per cent; the platelets numbered 239,000 per cubic millimeter; clotting time was three minutes and forty seconds, and bleeding time was three minutes. The red blood cells appeared normal in size and hemoglobin content. Blood cultures and agglutination tests for typhoid, paratyphoid and undulant fever were negative. The sedimentation rate remained rapid. The patient stayed in the hospital for one week, two days of which she was febrile, with maximum elevations of temperature of 101 and 102 F.

From the Department of Pathology, University of Pittsburgh, and the Presbyterian Hospital.

1. The term "myelophthisic" has been applied to this type of anemia.

The third admission was on September 5, because of severe urgency and frequency of urination. Retrograde pyelography revealed slight dilatation of the right renal pelvis. The urine from the right kidney contained many white cells, and culture yielded Bacillus coli communior. The red blood cell count varied between 2,500,000 and 3,400,000; the hemoglobin content, from 50 to 80 per cent. The results of additional laboratory tests were normal. Treatment during the three week period in the hospital consisted of ureteral catheterization of the right kidney and two transfusions of whole blood. During this period a remittent fever with peaks of 101 to 102 F. was recorded.

The fourth hospital admission was on October 4, for cystoscopic treatment. The physical findings remained normal. The red blood cell count was 3,500,000; the hemoglobin content was 68 per cent. She was discharged after three days. The highest recorded temperature in this period was 102.4 F.



Section of vertebral marrow showing diffuse granulomatous infiltration replacing the hemopoietic tissue. × 300.

The fifth and final admission was on October 18, for lethargy, listlessness and excessive fatigue, which became progressively more severe until death on December 19, nine months after the first admission. The anemia failed to respond to repeated blood transfusions, injections of a liver extract with added thiamine hydrochloride, liver, marrow extract and amigen.

During this admission tenderness over the lower border of the liver was noted. At no time was the spleen palpable. A cardiac murmur was heard at the apex on several occasions. A week before death icterus was noted, and moist rales were present in the middle and lower lobes of the right lung. The temperature was persistently intermittent with daily elevations to 102 to 103 F.

The laboratory findings during the last admission were as follows: The urine contained a trace of albumin; the specific gravity varied from 1.010 to 1.020. There was a progressive decline in the red blood cell count from 3,400,000 to 1,970,000; the hemoglobin content dropped from 70 to 41 per cent. The erythrocytes showed

slight anisocytosis and poikilocytosis. The reticulocytes were 3.4 and 6 per cent; the white blood cell count varied from 1,000 to 4,000, with the percentage of mature polymorphonuclear leukocytes ranging from 58 to 70. Serum protein was 4.5 Gm. per hundred cubic centimeters, with albumin 3.9 Gm. and globulin, 0.6 Gm. Blood cultures were repeatedly negative.

Autopsy (twelve hours after death).—External examination revealed nothing noteworthy except moderate malnutrition and slight icteric discoloration of the skin and the scleras. The cervical, axillary and inguinal lymph nodes were not enlarged.

The lungs showed passive congestion. In the lower lobe of the left lung there were irregular areas of pneumonic consolidation containing miliary foci of suppuration.

The heart was grossly normal.

The spleen was moderately enlarged. It weighed 465 Gm. and measured 15 by 10 by 6 cm. On section the pulp was dark red and studded with pale, slightly elevated fibrous nodules from 1 to 4 mm. in diameter.

Section through the liver revealed numerous small irregular pale infiltrations and circumscribed miliary nodules. There were a few larger lesions up to 1 cm. in diameter.

The peripancreatic and paravertebral lymph nodes were slightly enlarged, the largest measuring 3 cm. in greatest diameter. They were not adherent to one another. On section the tissue was pale, glistening, solid and of uniform appearance throughout.

The marrow of the lumbar and dorsal vertebrae, the ilium and the ribs was mottled in appearance, presenting extensive pale, glistening, soft fibrous areas intermingled with smaller irregular deposits of red marrow. The cortex in all areas was intact, and there was no apparent destruction of the spongiosa.

Microscopic Examination.—The lungs contained confluent areas of bronchopneumonia; several of the bronchioles were distended with purulent exudate.

In the myocardium there were a few small sharply circumscribed embolic abscesses.

The spleen contained numerous granulomatous nodules composed of coarse interlacing deposits of collagen containing small numbers of inflammatory cells of various types and scattered mononuclear and giant cells of the Sternberg-Reed type. Present throughout the intervening splenic pulp were similar cells in greater number.

A similar infiltration was noted throughout the liver; at times it resulted in coarse nodulations which had replaced areas of hepatic parenchyma.

Several of the lymph nodes showed extensive alteration of structure. There were considerable fibrosis and moderately heavy diffuse infiltration of lymphocytes, plasma cells, monocytes, and scattered giant cells with abundant acidophilic cytoplasm and oval lobate vesicular nuclei, frequently multiple. In areas eosinophilic leukocytes were numerous. Areas of ischemic necrosis, so characteristic of the advanced lesions of Hodgkin's disease, were not observed grossly or microscopically in any of the lymph nodes.

Sections of vertebrae, ilium and ribs revealed extensive granulomatous infiltrations which had largely replaced the hemopoietic tissue. Only occasional irregular islands of uninvolved marrow were observed.

Various types of inflammatory cells and giant cells of the Sternberg-Reed type were present throughout a cellular fibroblastic matrix. The bony trabeculae were essentially normal in appearance; no destruction of bone was evident. There

were localized areas of ischemic necrosis of the granulomatous tissue traversed by unaltered lamellas of bone.

Sections from the esophagus and the adrenal gland revealed minimal infiltration of Hodgkin's type. Small embolic abscesses were seen in the kidneys.

COMMENT

The widespread involvement of the bone marrow resulting in extensive replacement of the hemopoietic tissue was undoubtedly responsible for the severe and progressive anemia, the most significant feature of the clinical picture. The infiltration was of such character that it did not cause destruction of bone and, therefore, was not productive of the deep-seated bone pain so typical of the osteolytic lesions of Hodgkin's disease.

Without a representative portion of the marrow for histologic study the diagnosis could not be made clinically in view of the vague clinical features and the nonspecific laboratory findings. Even at postmortem examination the exact nature of the disease was not apparent on gross inspection, because of the minimal enlargement of the lymph nodes.

The extent of the process in the marrow stands in striking contrast to the relatively minor involvement of the lymph nodes. One might question the orthodox interpretation that the lesion of Hodgkin's disease always commences in the lymph nodes. However, until cases of Hodgkin's disease with lesions confined to the marrow are described, the involvement of bone seen in this case must be considered secondary to a process primary in lymphoid tissue.

SUMMARY

An unusual case of Hodgkin's disease with extensive infiltration of the hemopoietic marrow resulted in severe and progressive anemia of the myelofibrotic type.